1988

An Assessment of Tributyltin Contamination in Sediments and Shellfish in the Chesapeake Bay

Francois A. Espourteille
College of William and Mary - Virginia Institute of Marine Science

AN ASSESSMENT OF TRIBUTYLTIN CONTAMINATION IN SEDIMENTS AND SHELLFISH IN THE CHESAPEAKE BAY

A THESIS

Presented to

The Faculty of the School of Marine Science

Virginia Institute of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

---

by

Francois A. Espourteille

1988

APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

Master of Arts

François A. Espourteille, Author

Approved, June 6 1988

Robert J. Huggett, Ph. D., Chairman

Michael E. Bender, Ph. D.

John D. Boon, III, Ph. D.

John Greaves, Ph. D.

Charlotte P. Mangum, Ph. D.

DEDICATION

I would like to dedicate this work to my maternal grandparents, Mr. and Mrs. Galbe, whose moral support and uncompromising love made life enjoyable. I would also like to thank them for creating a sense of permanence in an ever changing world. My only regret is that they are not with us to see this day.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. R. J. Huggett for awakening my interest to Chemical Oceanography and for his continuing support over the course of this research. I would also like to acknowledge the other members of my committee: Drs. M. E. Bender, J. D. Boon III, C. P. Mangum; Dr. J. Greaves deserves special thanks for his extensive and incisive reviews of this thesis.

I would like to express my gratitude to my co-workers who made laboratory routine bearable: Mr. H. Slone and Mr. J. Greene offered invaluable help during sample collection and Mrs. E. Travelstead provided incessant help with daily work.

I am grateful to Ms. E. Harvey for keeping the computer system and the GC equipment functional. Thanks are due to Dr. M. Unger for manufacturing the tripentyltin chloride standard.

Finally, I would like to thank my parents for their constant support throughout my life and without whom this work would not have been possible. Last but not least, I am grateful to my wife, Susan, for her patience and caring.

LIST OF TABLES

LIST OF FIGURES

AN ASSESSMENT OF TRIBUTYLTIN CONTAMINATION IN SEDIMENTS AND SHELLFISH IN THE CHESAPEAKE BAY

ABSTRACT

A method for the analysis of butyltin compounds from sediments and biota is described. Butyltins are hexane extracted from the samples and then reacted with the Grignardreagent, n-hexyl magnesium bromide. Products, tetraalkyltins, are separated and quantified by gas chromatography with flame photometric detection and confirmed by gas chromatography mass spectrometry.

Sediment and biota samples were collected for tributyltin analysis from the James, Wicomico, Rappahannock, Elizabeth and Lafayette Rivers and on the Eastern Shore of Virginia. Sediment samples only were collected in Hampton Roads. These sampling sites from the lower and middle Chesapeake Bay were chosen to include areas of economic, recreational and human importance.

Results revealed high (> 100 ppb) tributyltin concentrations in sediments from Hampton Roads and the Elizabeth River. Moderate tributyltin concentrations (between 50 and 100 ppb) were found in sediments from the James River, Wicomico River and the bay side of the Eastern Shore. Sediments from Rappahannock River and the ocean side of the Eastern Shore contained low tributyltin concentrations (< 10 ppb).

Very high tributyltin concentrations (> 1000 ppb) were encountered in biota samples from the Elizabeth River. High tributyltin concentrations (between 500 and 1000 ppb) were found in James River and Wicomico River biota. Biota samples from both the Rappahannock River and the bay side of the Eastern Shore of Virginia contained moderate tributyltin concentrations (between 100 and 500 ppb). Low tributyltin concentrations (< 100 ppb) were found on the ocean side of the Eastern Shore of Virginia.

An attempt is made at predicting the duration of the period during which tributyltin will be found in the sediments based on the data collected and its degradation rate.

AN ASSESSMENT OF TRIBUTYLTIN CONTAMINATION IN SEDIMENTS AND SHELLFISH IN THE CHESAPEAKE BAY

INTRODUCTION

1-1 GENERAL INFORMATION

1-1.1 Historical Background.

Organic compounds of tin, otherwise known as organotins, usually possess at least one and up to four carbon atoms covalently bonded to the tin atom. Organotins usually have tin in the IV+ oxidation state, even though a few compounds exist which have tin in other oxidation states. Penta and hexacoordinate complexes of tin exist (Laughlin and Linden 1985). The first organotin compound, diethyltin diiodide, was prepared by Sir Edward Frankland in 1849 (Neuman 1970). Organotins first became commercially significant in the 1940's when diorganotins, such as dibutyltin dilaureate, were found to stabilize polyvinyl chloride (PVC). Thiotin compounds were found to have properties similar to those of diorganotins, but were more efficient as heat stabilizers for PVC. In 1955 non-toxic octyltin stabilizers for PVC were discovered and used in food contact applications. Triorganotins in pesticides appeared in 1957 as the fungicide Brestan (triphenyltin acetate). They have been used since the 1960's in wood preservatives, and antifouling paints

(Evans and Karpel 1985). Bis(tributyltin) oxide was one of the first organotins to be used in antifouling paints in the early 1960's (Bennett and Zedler 1966).

1-1.2 Organotin Antifouling Paints.

Organotin antifouling paints usually contain tributyltin (TBT) as a biocide. TBT paints are available in two basic forms; conventional or free association paints and copolymer paints. Free association paints do not chemically bind the biocide. Release rate of the TBT into the surrounding water decreases exponentially over time. These paints usually leach much more biocide than needed at first and eventually leach too little to be effective. As a result the biocide level in the environment is significantly greater at the beginning of the boating season than at the end. Copolymer paints, the other basic form, theoretically leach the biocide at an almost constant rate after a short initially high release. TBT is chemically bound in the matrix of the paint and release occurs through hydrolysis of the tributyltin methacrolate units at the paint surface. Copolymer release rates are, in general, half those of conventional paints.

1-1.3 Toxicology of Triorganotins.

Alzieu et al. (1980 and 1982) and Alzieu and Heral (1984) were amongst the first to investigate the toxic effects of TBT used in antifouling paints. They studied the production decline of the French oyster industry linking it to shell malformation in adults and reduction in larval setting. These adverse effects increased with marina proximity. Laboratory tests validated the field observations. They suggested that TBT i) interferes with copper and zinc metabolism, ii) complexes with amino-acids that compose the proteic matrix of the shell and iii) inhibits oxidative phosphorylation.

While no other reference was found on the possible relationship between TBT and the copper and zinc metabolism, Smith (1978) found that triorganotin compounds are capable of binding to amino acids and dipeptides. More precisely they combine with the thiol and amidazole groups, thus adversely affecting the proteins. Saxena (1987) reported that the majority of organotins derive their toxicity from their ability to bind irreversibly to the active sites of enzymes, thus inhibiting pathways.

The toxic aspect of TBT as it relates to oxidative phosphorylation, suggested by Alzieu et al. (1980, 1982 and 1984), was specifically investigated by Blunden et al. (1984). They reported that the toxic activity of triorganotin compounds rests in their ability to derange

mitochondrial function, according to the following mechanisms: (1) triorganotin compounds interact with mitochondrial membranes causing swelling and disruption; (2) they interfere with oxidative phosphorylation by acting as ionophores and thus mediating of Cl^-/OH^- exchange across the lipid membrane, which inhibits fundamental energy conservation processes involved in the synthesis of ATP from ADP.

Hall and Pinkney (1985) assessed organotins to be more toxic to aquatic biota than polynuclear aromatic hydrocarbons (PAH), chlorinated insecticides, and polychlorinated biphenyls (PCB). Steinhauser et al. (1985) determined that, among organotins, triorganotins, which include TBT, are the most toxic to marine invertebrates.

1-1.4 Detection Methods for Organotins.

A major difficulty in studying organotins in the environment rests in detecting them. TBT concentrations in water as low as a few parts per trillion have been shown to have deleterious effects to marine life (Thain and Waldock, 1985; Hall et al. 1988). With such minute concentrations bearing significant importance, very sensitive analytical chemical and detection methods were required. Most organotin analyses can usually be divided into two groups: the hydride formation method and per-alkylation method by means of a Grignard reaction.

The hydride formation method is the older and involves the derivatization of organotins into organotin hydrides. Compound determination can be achieved, following hydride generation, in a variety of different ways. Donard (1983) separated organotin species on chromatographic packing material prior to detection with an electrothermal quartz furnace coupled to an atomic adsorption spectrophotometer. Absolute detection limits were good, 0.06 ng for methyltins and 0.025 ng for n-butyltins (expressed as tin). This method allowed for speciation, quantification and positive identification of organotin species. Another detection procedure involved analyzing organotin hydrides with a gas chromatograph coupled to a mass spectrometer, which also speciated, quantified and identified organotins (Gilmour, Tuttle and Means, 1986; Hodge, Seidel and Goldberg, 1979). Matthias et al. (1985) used gas chromatography with flame photometric detection and obtained detection limits of 7 ng Sn/L for TBT and 3 ng Sn/L for dibutyltin for 100 ml samples. Tsuda et al. (1986) used gas chrcmatography with electron capture detection to analyze organotins and claimed to obtain detection minimums of 1 to 2 ng/g for biota and 0.5 to 1 ng/g for sediments. However, they only tested samples fortified with 50 ng/g organotin.

The Grignard reaction method, the other basic type, results in the per-alkylation of organotins. Maguire and Tkacz (1983) compared the effectiveness of gas chromatography with flame photometric detection and gas chromatography with atomic adsorption spectrophotometry. Sensitivity did not differ much between either methods, but they found the latter equipment to be simpler and cheaper to maintain. Mueller (1984) and Unger et al. (1986) both used gas chromatography with flame photometric detection to analyze organotins and gas chromatography with mass spectrometry to verify the identity of the different species.

1-2 TRIBUTYLTIN IN SEDIMENTS

1-2.1 General Considerations.

TBT contamination in lake and coastal waters (Maguire et al. 1982; Mueller, 1984; Valkirs et al. 1985; Unger et al. 1986) has caused concern about the potential contamination of the underlying sediments. Toxicants in the water column will often sorb to particles in the sediments and can reach concentrations orders of magnitude greater than those found in the water above. While sediments can act as a sink for toxicants, sediments can also act as source of toxicants if the gradient is reversed (e.g. decreased or eliminated TBT in the water).

1-2.2 Analyses of Organotins in Sediments.

Analyses of organotins in sediments have been performed in the past, but some of the analytical methods used were such that the data may be questionable. For example, Hodge, Seidel and Goldberg (1979) oven dried sediments at 110°C before TBT extraction and analysis. This can result in 60 % TBT loss, most likely due to volatization (Rice, Espourteille and Huggett, 1987). Maguire (1984) developed a methodology which required air drying the sediments prior to extraction. No indications were given concerning the fate of TBT during the drying procedure, which was lengthy. Maguire et al. (1985) analyzed sediments which were freeze dried prior to extraction. This can also cause loss of TBT (Rice, Espourteille and Hugget, 1987). Maguire et al. (1986) reported on another set of sediment analyses without explaining how the samples were dehydrated, thus making interpretation of the results difficult. Mueller (1984) used a different method. TBT was transformed to tributylmethyltin, a very volatile compound, which could result in serious TBT loss during sample concentration. Tsuda et al. (1986) determined TBT in biological samples by adding concentrated HCl, thus driving most of the TBT^+ to TBTCl, and subsequently extracted the samples with a mixture of ethyl acetate and hexane. They extracted sediments in the same fashion, but used only n-hexane as

the extraction solvent. The resulting organic phase was reacted with sodium borohydride to form the tin hydrides. The samples were then purged and analyzed by gas chromatography. This method has two disadvantages: First, hydrides of tin must be analyzed soon after extraction and cannot be stored for long periods of time. Second, they claimed detection limits of 0.5 to 1 ppb for sediment samples but only tested spiked samples in 50 ppb range, thus casting doubts on this lower limit. It would seem they interpolated to obtain the detection limit without actually testing it.

To overcome some of the above stated problems it was important to develop a reliable method to analyze TBT in sediments.

1-3 TRIBUTYLTIN IN INVERTEBRATES

1-3.1 Tributyltin Toxicity to Marine Invertebrates.

TBT has been proven harmful to some marine organisms, specifically invertebrates (Becerra-Huencho, 1984; Beaumont et al. 1984; Laughlin et al. 1984). Laughlin, Nordlund and Linden (1984) showed TBT compounds to be slow acting toxins at low experimental concentrations.

Larval stages of these organisms are particularly sensitive to TBT (Laughlin et al. 1983). Roberts (1987) reported the LC_{50} (48 hr tests) for Merceneria merceneria and Crassostrea virginica embryos to be 1.13 ug/l and

1.30 ug/l, respectively. Differential larval survival of the amphipod, Gammarus oceanicus, occurred when exposed to 0.3 ug/l TBTO over an eight week period (Laughlin, Nordlund and Linden 1984). Zoeae of the mud crab, Rhithropanopeus harrisii, displayed differential mortality when exposed to 10 ug/l TBTO and 20 ug/l tributyltin sulfide (TBTS) (Laughlin, French and Guard 1982). Thain and Waldock (1985) observed reduced weight gain in Ostrea edulis spat when exposed to 0.06 ug/l TBTO under static conditions. TBTO concentration of 2.6 ug/l completely inhibited growth.

Chronic effects due to long term exposures of adults have been documented (Alzieu and Heral, 1984). Acute toxicity experiments showed that TBT presents environmental hazards to some adult invertebrates in the parts per trillion range. The copepod, Acartia tonsa, died within six days when exposed to 0.3 ug/l TBTO (U'Ren 1983).

Gibbs and Bryan (1986) reported on a peculiar problem caused by TBT. The common dogwelk, Nucella lapillus, found on the southwest coast of England exhibited numerous cases of imposex, or the superimposition of male characteristics (such as a penis and vas deferens) on the reproductory apparatus of female animals. The number of imposex cases in the population increased in waters with extensive boating. The incidence of imposex also

correlated with the TBT concentrations in tissues. TBT is believed to be responsible for the decline of the dogwelk.

A summary of the toxicity of TBT to many aquatic organisms is provided by M. Rexrode (1987); he lists gastropods and bivalves as being the most sensitive species (0.02-0.14 ppb), these are followed by crustaceans (0.09-0.14 ppb), algae (0.1-0.35) and finally fish ($\geq$ 0.2 ppb).

1-3.2 Tributyltin Data for the Chesapeake Bay.

The Chesapeake Bay is the largest estuary in the United States. It boasts an important shellfish industry along with a valuable multispecies finfishery. Hall et al. (1988) found estuarine copepods from the Chesapeake Bay (Eurytemora affinis) to be highly sensitive to low concentrations of TBT. 72 hr LC50 at 0.6 ug/L and 48 hr LC50 at 2.2 ug/L for E. affinis were recorded. Hall et al. (1988) also reported reduced survival of neonates when exposed to 100 ug/l TBT for 6 hr and in a chronic test which showed significant adverse effect to the copepods at the 0.2 ug/l range. Many locations near marinas within the Chesapeake Bay registered TBT concentrations in excess of these values (Huggett et al. 1986, Westbrook et al. 1986).

1-3.3 Tributyltin and Shellfish.

Oysters, Crassostrea virginica, and clams, Merceneria merceneria, account for a large part of shellfish landings in the bay area. Such bottom dwelling filter feeders are capable of bioaccumulating TBT from their environment by a factor of one thousand or more (Hall and Pinkney 1985). Bioaccumulation factors of 60,000 have been observed in the laboratory (Bender, personal communication). This is due, in part, to their limited ability to metabolize the compound (Lee, 1986). Furthermore, clams are found in sediments which are nearly anoxic. Such sediments may lack the bacteria necessary to degrade TBT, thus exposing these animals to high levels of toxicant (Gilmour, Tuttle, and Means 1986). Many adult mollusks, while capable of surviving high environmental levels of TBT can be chronically affected though scallops, Pectens maximus, appear to be an exception (Paul and Davies, 1986).

Preliminary research suggests that low lipid reserves in oysters are related to high TBT concentration in tissues. Such a concept agrees with the conclusion of Blunden et al. (1984) that TBT interferes with intracellular energy yielding pathways. Lipid poor oysters are likely to be more susceptible to environmental stresses and parasites. Laughlin, French, and Guard (1986) and Evars and Laughlin (1984) have found that mussels Mytilus edulis and the mud crab R. harrisii

bioaccumulate TBT. Shellfish contaminated with TBT can expose humans to the compound. The effects, if any, of such exposures are unknown at this time. Analysis of shellfish from commercial production areas would provide information on exposure of humans to TBT as well as provide an insight into the exposure of the animals themselves to the compound.

1-4 FOCUSSING ON CONTEMPORARY PROBLEMS

1-4.1 Tributyltin in Sediments and Biota in the Chesapeake Bay: Current Evidence.

At this point in time, limited data are available on TBT concentration in sediments of the Chesapeake Bay. Several papers reported on TBT concentrations in sediments. Values ranged from 141 to 1,390 ug/kg dry weight from Back Creek, northern Chesapeake Bay (Matthias et al.). Also Severn River sediments were found to contain 50 ug/kg near Back Creek. Two other reports originated from the Virginia Institute of Marine Science and listed sediment values ranging from 23 to 290 ug/kg dry weight (Sarah Creek, King Creek; Virginia) (Rice, Espourteille and Huggett, 1987) and 920 to 1300 ug/kg dry weight (Sarah Creek and Hampton Roads; Virginia) (Westbrook et al. 1986). The method used was the one developed at this institute.

Only one paper was found to report on TBT concentration in Chesapeake Bay biota; organisms analyzed were American oysters Crassostrea virginica from Sarah Creek (a York River tributary) with concentrations averaging 834 ± 430 ug/kg wet weight (Rice, Espourteille and Huggett, 1987).

1-4.2 Objectives.

Several benefits could be derived from better defining the extent of TBT contamination in sediments and tissues. Decreases in oyster landings, quality and or reproduction have been blamed on known problems such as MSX. MSX is generally accepted as the cause for the 1960 decline in oyster yield. MSX along with chlorinated sewage effluents (Haven, Hargis and Kendall, 1978) have been blamed for low levels of larvae in the James River between 1965 and 1972. TBT appeared in antifouling paints in 1966 (Bennett and Zedler, 1966), and could have augmented a process started by MSX. Roberts (1987) concluded that TBT is highly toxic to embryos and larvae of both C. virginica and M. merceneria. Better knowledge of TBT pollution could enable us to understand whether this compound plays a role in the problems facing the bivalve fisheries.

The Chesapeake Bay is polluted with TBT (Unger et al., 1986; Huggett et al., 1986; Rice, Espourteille and Huggett, 1987). Knowledge of the exact extent of this pollution is necessary. If unacceptably high levels of TBT

are encountered either in mollusks or sediments, action may be necessary to restrict fishing and sale of oysters and clams. Once the extent of the pollution is recognized, it may be possible to evaluate solutions and forecast a time frame within which TBT will remain a problem, provided TBT sources are eliminated or greatly reduced.

EXPERIMENTAL

2-1 METHOD DEVELOPMENT

2-1.1 Introduction.

Unger et al. (1986) reported on a methodology for the analysis of butyltins in water. The sediment methodology used here closely follows that of water with one notable exception- removing TBT from the matrix. Extraction of TBT from water is relatively simple, whereas sediments tend to bind organotins tightly making extraction more difficult.

The solvent used for water extraction, n-hexane, is nonpolar and cannot efficiently remove compounds sorbed onto clay particles which are surrounded by a hydration sheet. Changing to a polar solvent (such as acetone, methanol or diethyl ether) is impractical due to potential reactions with the Grignard reagent. Most polar solvents react with Grignard reagents and are therefore unsuitable. The alternative was to dehydrate sediments prior to extraction. Oven drying for 12 hours at 50^{o} C or freeze drying for 24 h (-20^{o} C to 35^{o} C) were proven inappropriate due to significant loses of organotins (Table 1), while air drying was deemed too lengthy.

Table 1.

TRIBUTYLTIN LOSS DUE TO SAMPLE DEHYDRATION
BY FREEZE DRYING OR OVEN DRYING

SAMPLE	TBT+ SPIKE (ppb)	RECOVERED TBT+ (ppb)	% LOSS
1	50	19	62
2	50	20	60
3	50	17	66
4	50	17	66
5	50	16	68
6	50	15	70

50 ppb of TBT+ were added to each sample before dehydration. Samples 1-3 were freeze dried for 24 h (temperature range -20^{o} to 15^{o} Celcius, vacuum 350 mTorr), samples 4-6 were oven dried for 12 h at 50^{o} Celcius.

2-1.2 Sample Preparation.

As an alternative to thermal drying, chemical desiccation was employed. A 10 g wet sediment subsample was placed in a clean quart glass jar (scrubbed with Contrad, rinsed with distilled water, dilute HCl, acetone and hexane). In addition a second subsample was taken and oven dried at 100^{o} C overnight to determine total moisture since dry weight organotin concentrations were usually desired. Prior to desiccation, a known amount of tripentyltin chloride was added to the sediment as an internal standard. The exact amount of internal standard added depended upon the expected sediment concentration and should be within a factor of five of the actual TBT concentration to keep the error due to comparing the GC responses within acceptable limits. Testing has shown that as the disparity between peaks to be compared grows, so does the quantification error (from about 5% to 30% or more). Tripentyltin chloride was synthesized in the laboratory, as described by Unger et al. (1986).

The sediment samples were dried with three parts desiccant to one part sediment. The desiccant mixture consisted of 10% by weight QUSO G35 (precipitated silica, DeGussa Corporation) and 90% by weight anhydrous granulated sodium sulfate (Fisher Scientific Co.). QUSO G35 was blended in first, followed by sodium sulfate. The sample was then frozen for one hour or more which

completed moisture removal from the sediment and also lysed cells present in the sample.

2-1.3 Sediment Methodology.

The sample was blended to a fine powder consistency using a blender head screwed onto the jar. The sample was then added to a glass soxhlet thimble with coarse frit, in which glass wool had been placed. The lower wool layer prevented frit clogging. Glass wool was added to the top of the sample to reduce sample spattering and carry over into the solvent reservoir. Whatman^{R} Cellulose thimbles were tried and found to contain variable levels of both tributyltin and dibutyltin and were therefore unacceptable. Concentrations as high as 29 ppm were found. Not all lots were contaminated.

The samples were soxhlet extracted for 48h with 400ml of n-hexane (American Burdick and Jackson). Shorter extraction time resulted in incomplete removal of tributyltin and dibutyltin; sediments extracted for only 24 h retained up to 16% of their organotins (Table 2). The extract was reduced to 5ml by roto-evaporation at 40^{O} C and transferred to a 50ml centrifuge tube. The original flask was rinsed three times with 5ml aliquots of hexane (final sample volume of the centrifuge tube was 20ml). The sample was subsequently derivatized with 0.5ml of n-hexyl magnesium bromide (2.0 M Aldrich Chemical Co.) for 30 minutes. During this time the sample was agitated

Table 2.

SOXHLET EXTRACTION OF TRIBUTYLTIN FROM NATURALLY CONTAMINATED SEDIMENTS

TBT+ concentration (in ppb) found after repeated extractions

TIMES IN HOURS

SAMPLE	24	48	72
1	870*	140	n.d.**
2	870	77	n.d.
3	900	32	n.d.
4	930	100	n.d.

* 10g sediment samples were soxlhet extracted for 24h, 48h, and 72h. Each time the extractant was analyzed for TBT and the same sediment sample reextracted.

** Not detectable at lppb.

every five minutes. Following the reaction which converted tributyltin, dibutyltin and tripentyltin to hexyltributyltin, dihexyldibutyltin and hexyltripentyltin, respectively, 2ml of concentrated HCl were added to remove excess Grignard reagent from the sample. All of the white precipitate was dissolved in order to free any derivatized TBT that may have been trapped by the particles. The resulting aqueous phase was discarded and the organic phase was reduced to 1ml under dry nitrogen at 40^{o} C. The sample was then cleansed by passing it through a 22mm i.d. chromatographic column packed with 25g of activated Florisil^{R} (100-200 mesh, Fisher Scientific Co.) topped with 2g of anhydrous sodium sulfate (Fisher Scientific Co.). The sample was then eluted with 300ml of n-hexane and roto-evaporated to 5ml. It was then transferred to a 50ml centrifuge tube and reduced under dry nitrogen to a volume suitable for GC analysis which was between 0.05-2.00ml in most cases.

2-1.4 Gas Chromatographic Analysis.

Analysis of the sample was performed on a Varian model 3300 gas chromatograph with a dual flame photometric detector with >600nm band pass filter. The column was a 20 m glass capilary 0.32 mm i.d., coated with 1 uSE-52, immobilized with dicumyl peroxide (Unger et al., 1986). The injector and detector were held at 275^{o} C, and the column temperature was programmed from 120^{o} C to 280^{o} C at

10^{o} C/min. Resting temperature of the column was 45^{o} C. Helium was used as the carrier gas, with a flow of 4 ml/min; hydrogen and air flow rates to the detector were 138 ml/min and 250 ml/min, respectively. Detector output was collected on a HP 3354B laboratory automation system which integrated the butyltin peaks and quantified them relative to the internal standard peak. Confirmation of the identity of organotins observed on the GC was carried out for selected samples on a gas chromatograph (Hitachi, Model 663-30)- quadrupole mass spectrometer (Extrel, Model EL-400-2). For more detailed information see Unger, et al. (1986).

2-1.5 Tissue Methodology.

Methodology for analysis of oyster tissues was developed concurrently to the sediment method by C. Rice and this author (Rice, Espourteille and Huggett, 1987). Differences between the two methods lay in the need to grind oyster tissue thoroughly (Virtis Homogenizer) to obtain a fluid homogenate prior to desiccation. Tissue samples also require a shorter extraction time (24h).

2-2 METHOD VALIDATION

In order to determine the accuracy and the precision of this method, two different tests were implemented. First, to determine the accuracy of the methodology, known

quantities of tributyltin chloride (20 ug/kg, 100 ug/kg, 500 ug/kg) were added to pristine sediments collected from Carter Creek, a small isolated tributary of the York River. Five replicates were carried out at each concentration. The internal standard was added after soxhlet extraction since absolute recoveries were desired. Second, to determine the precision of the method, one sample, assumed to contain tributyltin, from Sarah Creek (a tributary of the York River) was divided into 5 subsamples. Each subsample was analyzed as described above.

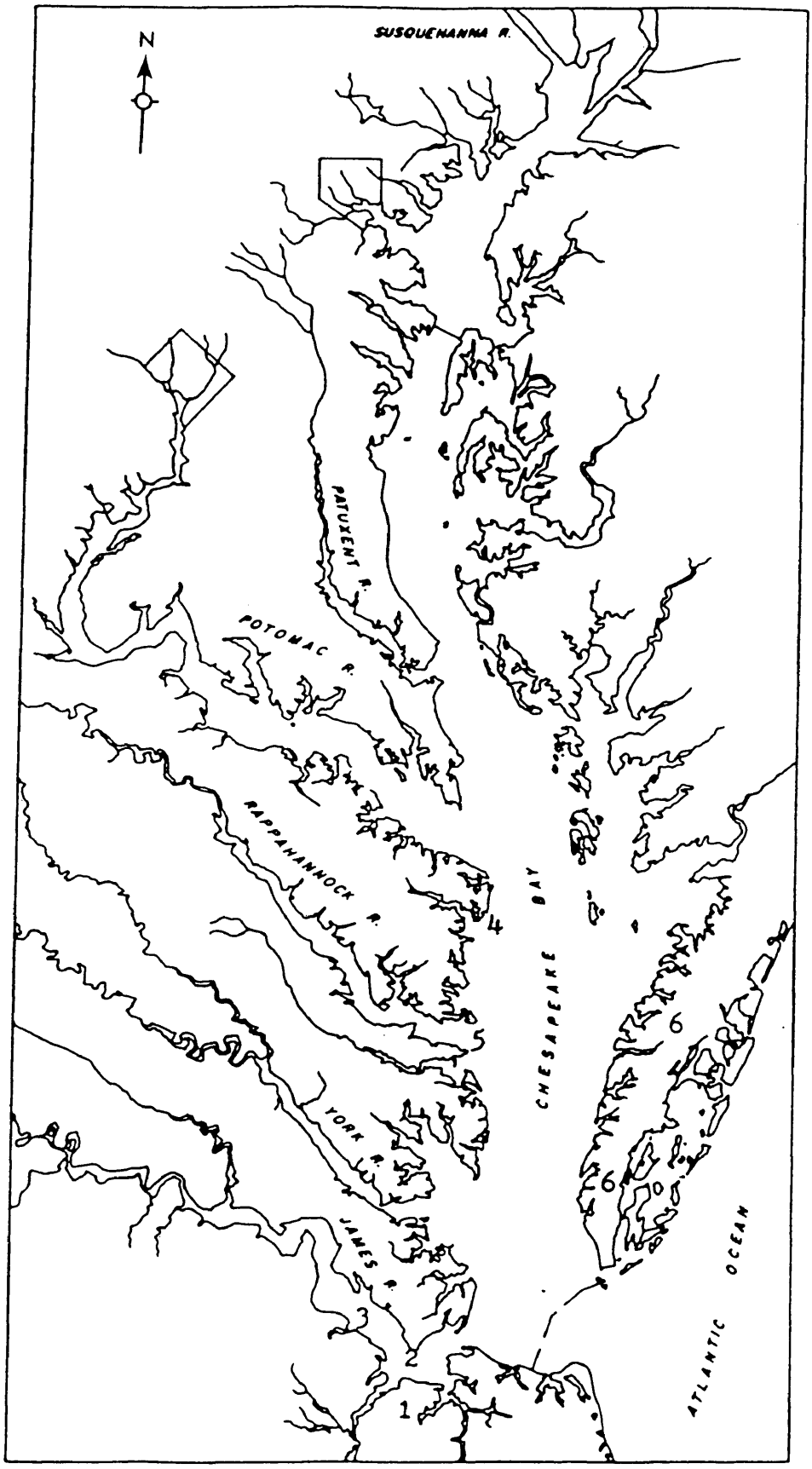
A third test was implemented to determine the TBT concentration variation in the sediments that can be encountered at one station. For this purpose, five sediment samples were taken in the Lafayette River at the marker number 13 and analyzed separately as described above.

2-3 COLLECTION AND TREATMENT OF SAMPLES

2-3.1 Sampling Sites.

The map of the Chesapeake Bay shown in figure 1. depicts the general sampling areas. The goal of this report was to assess the importance of TBT pollution in sediments and biota of the Chesapeake Bay, with particular interest being paid to areas of human and commercial

Figure 1. Map of the Chesapeake Bay showing the main sampling areas: 1- Elizabeth River; 2- Hampton Roads; 3- James River; 4- Great Wicomico River; 5- Rappahannock River; 6- Eastern Shores.


N
SUSQUEHANNA R.
PATUXENT R.
POTOMAC R.
RAPPAHANNOCK R.
YORK R.
JAMES R.
CHESAPEAKE BAY
ATLANTIC OCEAN
1
2
3
4
6
6
0

significance. To achieve this goal, it was decided to sample the major oyster rocks in the James River (figure 2), which is an important oyster breeding ground, the Rappahannock River (figure 3), which produces a large amount of marketed oysters, and the Great Wicomico River (figure 4) where some oyster harvesting still occurs. The Eastern Shore of Virginia (figure 5) was sampled both for its importance in oyster production and to assess TBT concentrations in these recreational areas. Hampton Roads, the Elizabeth River and the Lafayette River were sampled because they were believed to be highly polluted (figures 6 and 7).

2-3.2 Sediment Collection.

All sediment samples were taken with a ponar grab and only the top two centimeters of the undisturbed sediment column were collected. Some stations required that more than one grab be taken in order to obtain sufficient sample due to the sediment type (sandy, coarse shells). Samples were stored in the dark while in the field. Once returned to lab, they were kept at 12^{o} C for no longer than 3 days before being analyzed.

Figure 2. The James River is depicted. Sampling locations are shown: 1- Deep Water Shoal; 2- Horse Head; 3- Jail Island; 4- Point of Shoal; 5- Wreck Shoal; 6- Warwick River mouth; 7- Pagan River mouth; 8- White Shoal; 9- Thomas Rock; 10- Brown Shoal; 11- Naseway Shoal; 12- Nansemond Ridge.

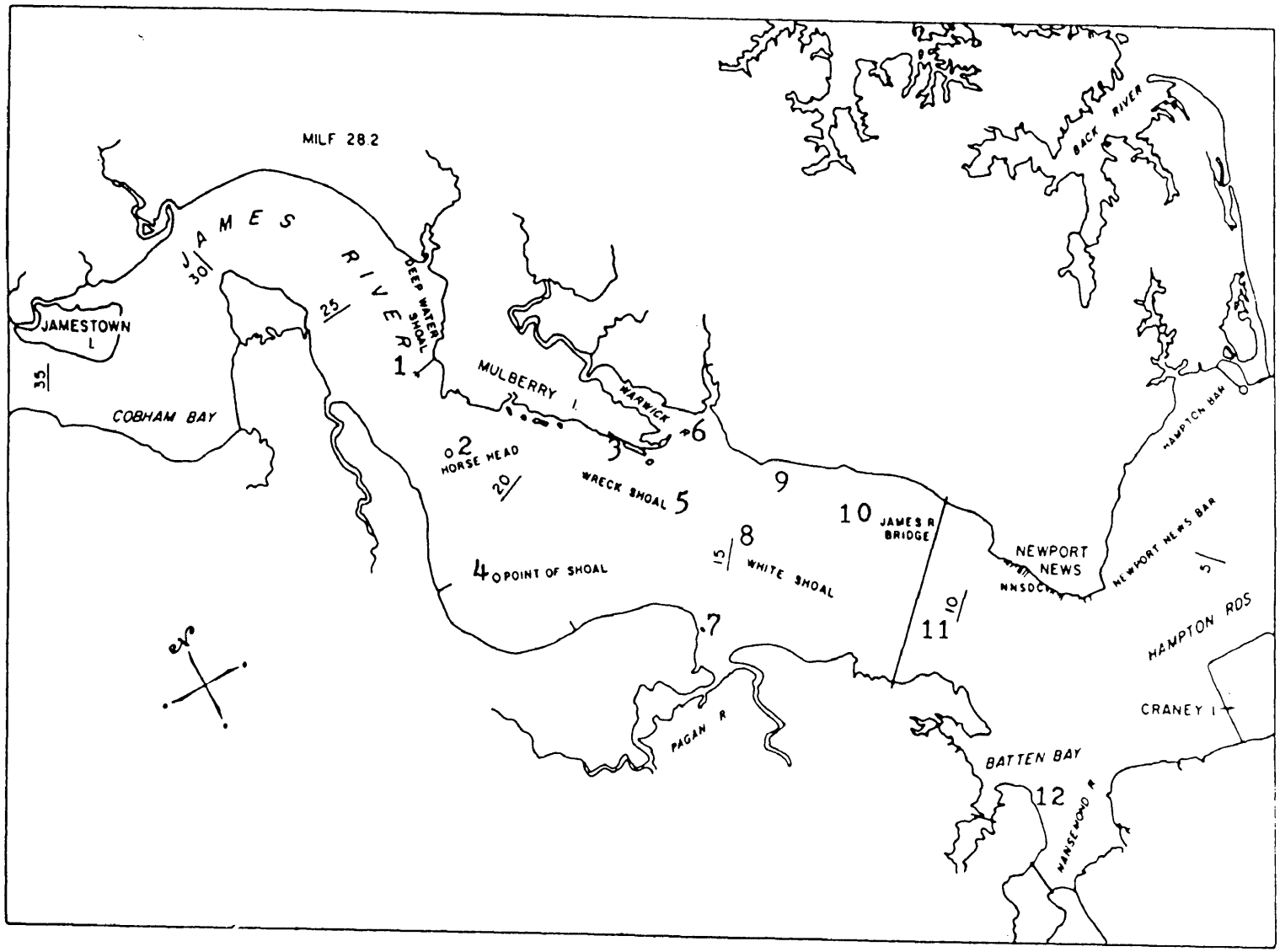

MILF 28.2
JAMES RIVER
JAMESTOWN I.
COBHAM BAY
DEEP WATER SHOAL
MULBERRY I.
WARWICK
HORSE HEAD
WRECK SHOAL
POINT OF SHOAL
WHITE SHOAL
JAMES R BRIDGE
NEWPORT NEWS
NNSDC
BACK RIVER
HAMPTON BAR
NEWPORT NEWS BAR
HAMPTON ROS
CRANEY I.
BATTEN BAY
PAGAN R
MANSEWOOD R
1
2
3
4
5
6
7
8
9
10
11
12
35
30
25
20
15
10
5

Figure 3. The Rappahannock is depicted. Sampling locations are shown: 1- Bowlers Rock; 2- Morattico Bar; 3- Belle Isle; 4- Monaskon Bluff; 5- Punch Bowl; 6- Smokey Point; 7- Hog House Bar; 8- Corrotoman River mouth; 9- Parrots Island.

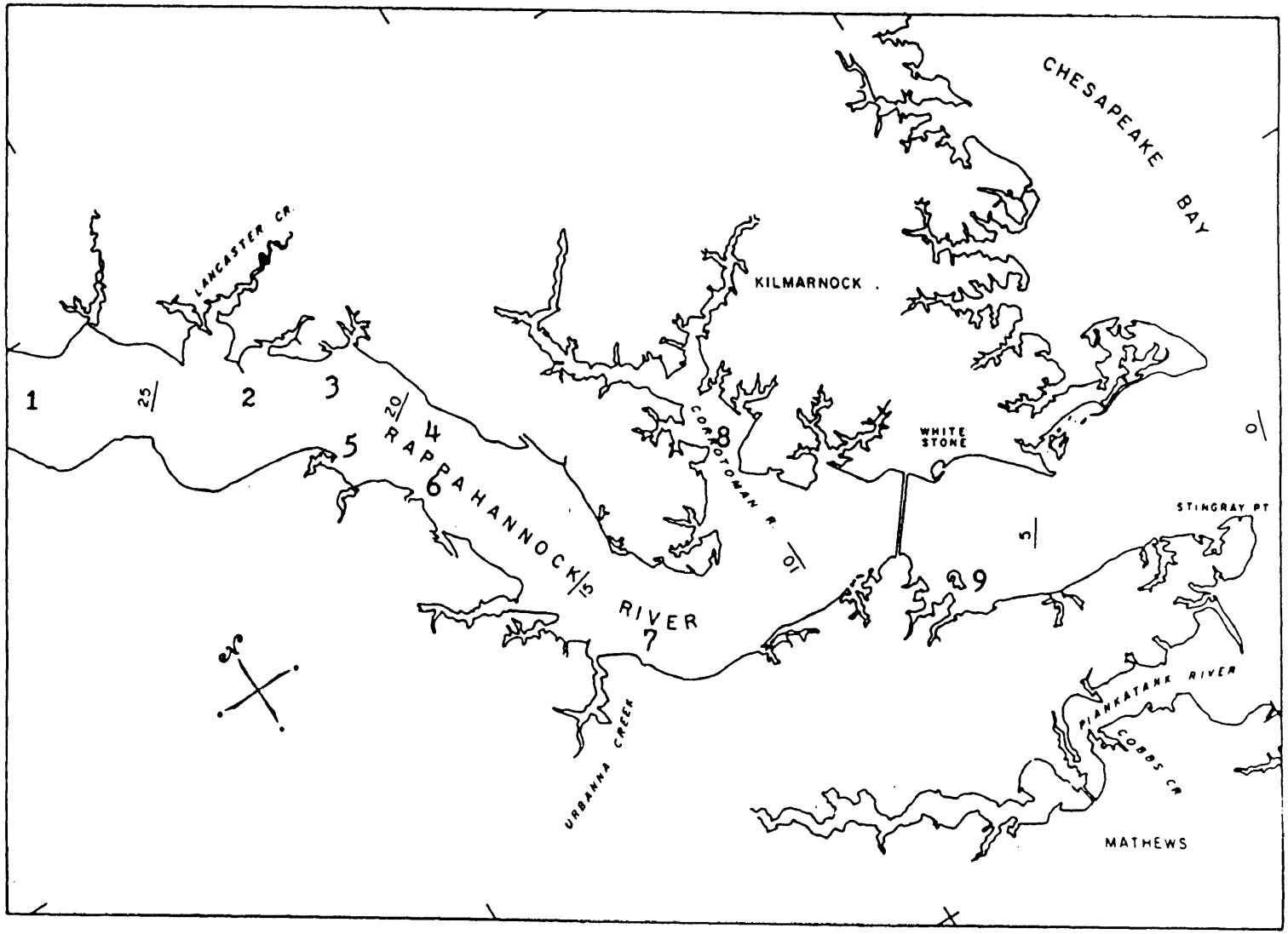

CHESAPEAKE BAY
LANCASTER CR.
KILMARNOCK
1
25
2
3
20
4
5
RAPPAHANNOCK
6
15
RIVER
7
COROTOMAN R.
8
10
WHITE STONE
0
5
STINGRAY PT
9
PIANKATANK RIVER
COBBS CR.
URBANNA CREEK
MATHEWS

Figure 4. The Great Wicomico River is depicted. Sampling locations are shown: 1- Glebe Point; 2- Rogue Point; 3- Haynie Point; 4- Bussel Point; 5- Fleet Point.

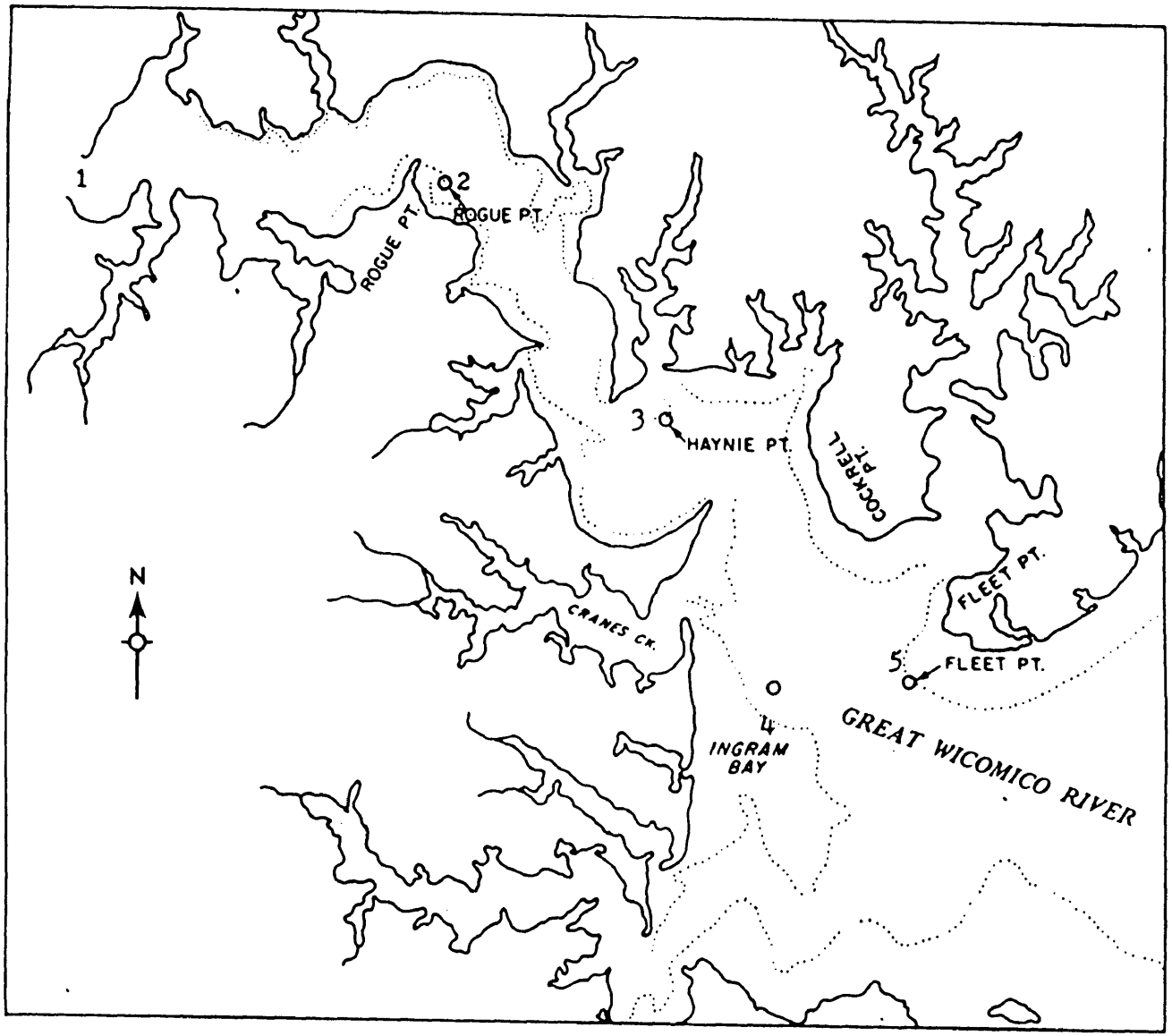

1
2
ROGUE PT.
ROGUE PT.
3
HAYNIE PT.
COCKRELL PT.
N
CRANES CK.
FLEET PT.
5
FLEET PT.
4
INGRAM BAY
GREAT WICOMICO RIVER

Figure 5. The Eastern Shore of Virginia is depicted. Sampling locations are shown: 1- Messongo Creek; 2- Onancock Creek; 3- Hacksneck; 4- Occahannock Creek; 5- Nassawadox Creek; 6- Hungars Creek; 7- Hungars Wharf; 8- Cherrystone Inlet; 9- Plantation Creek; 10- Oyster (town); 11- Cobb Bay; 12- Willis Wharf; 13- Wachapreague; 14- Metompkin, barrier island; 15- Folly Creek; 16- Metompkin, near shore; 17- Bogues Bay; 18- Chincoteague Bay (2); 19- Chincoteague Bay (1).

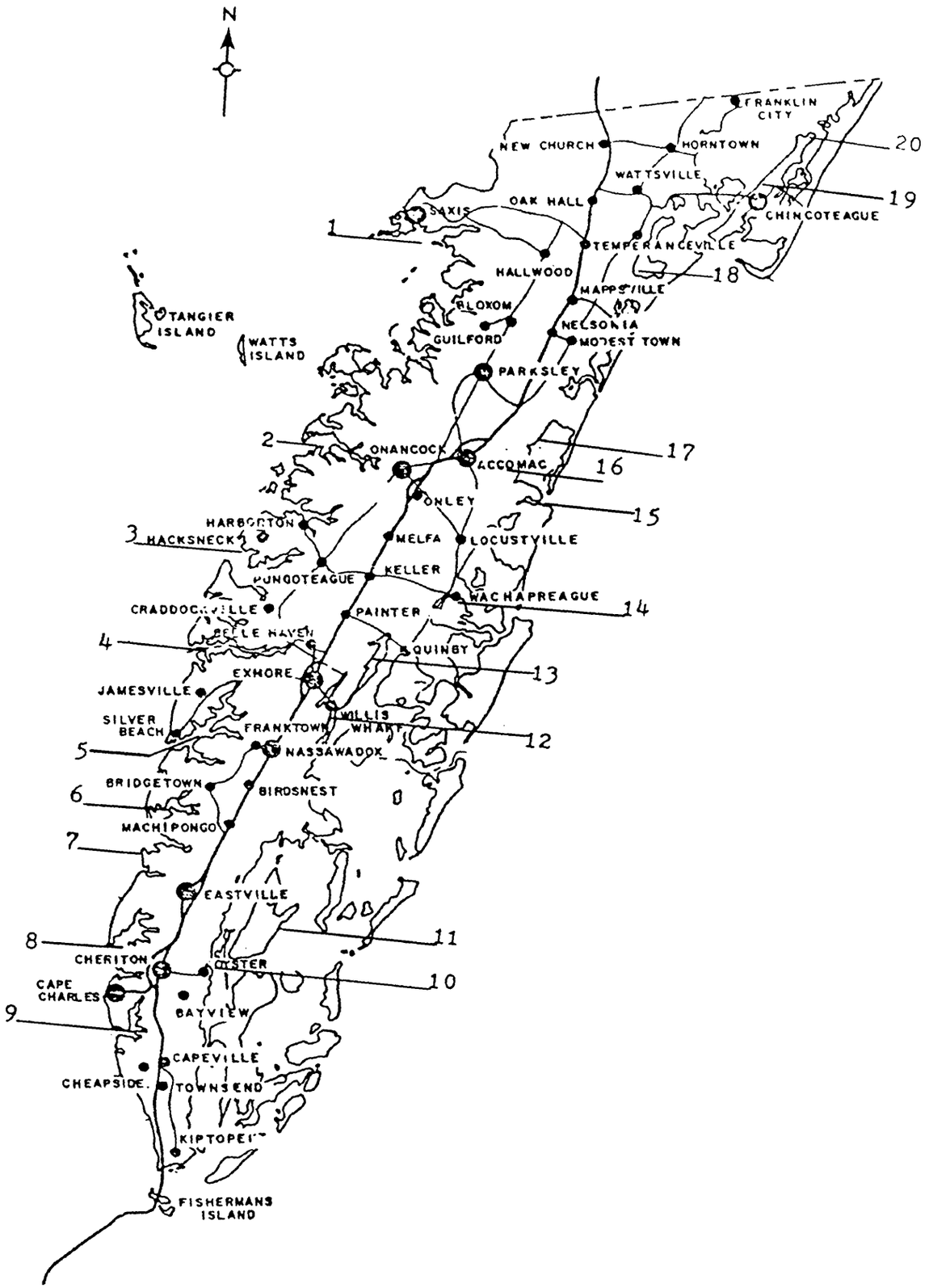

N
FRANKLIN CITY
NEW CHURCH
HORNTOWN
WATTSVILLE
OAK HALL
SAXIS
CHINCOTEAGUE
TEMPERANCEVILLE
HALLWOOD
MAPPSVILLE
BLOXOM
NELSONIA
MODEST TOWN
GUILFORD
TANGIER ISLAND
WATTS ISLAND
PARKSLEY
ONANCOCK
ACCOMAC
ONLEY
HARBORTON
HACKSNECK
MELFA
LOCUSTVILLE
KELLER
PUNGOTEAGUE
WACHAPREAGUE
CRADDOCKVILLE
PAINTER
BELLE HAVEN
QUINBY
EXMORE
JAMESVILLE
WILLIS WHARF
SILVER BEACH
FRANKTOWN
NASSAWADOX
BRIDGETOWN
BIRDSNEST
MACHIPONGO
EASTVILLE
CHERITON
CAPE CHARLES
BAYVIEW
CAPEVILLE
CHEAPSIDE
TOWNSEND
KIPTOPEKE
FISHERMANS ISLAND
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Figure 6. The Hampton Roads are depicted. Sediment samples only were taken at these sites (July and August 1986).

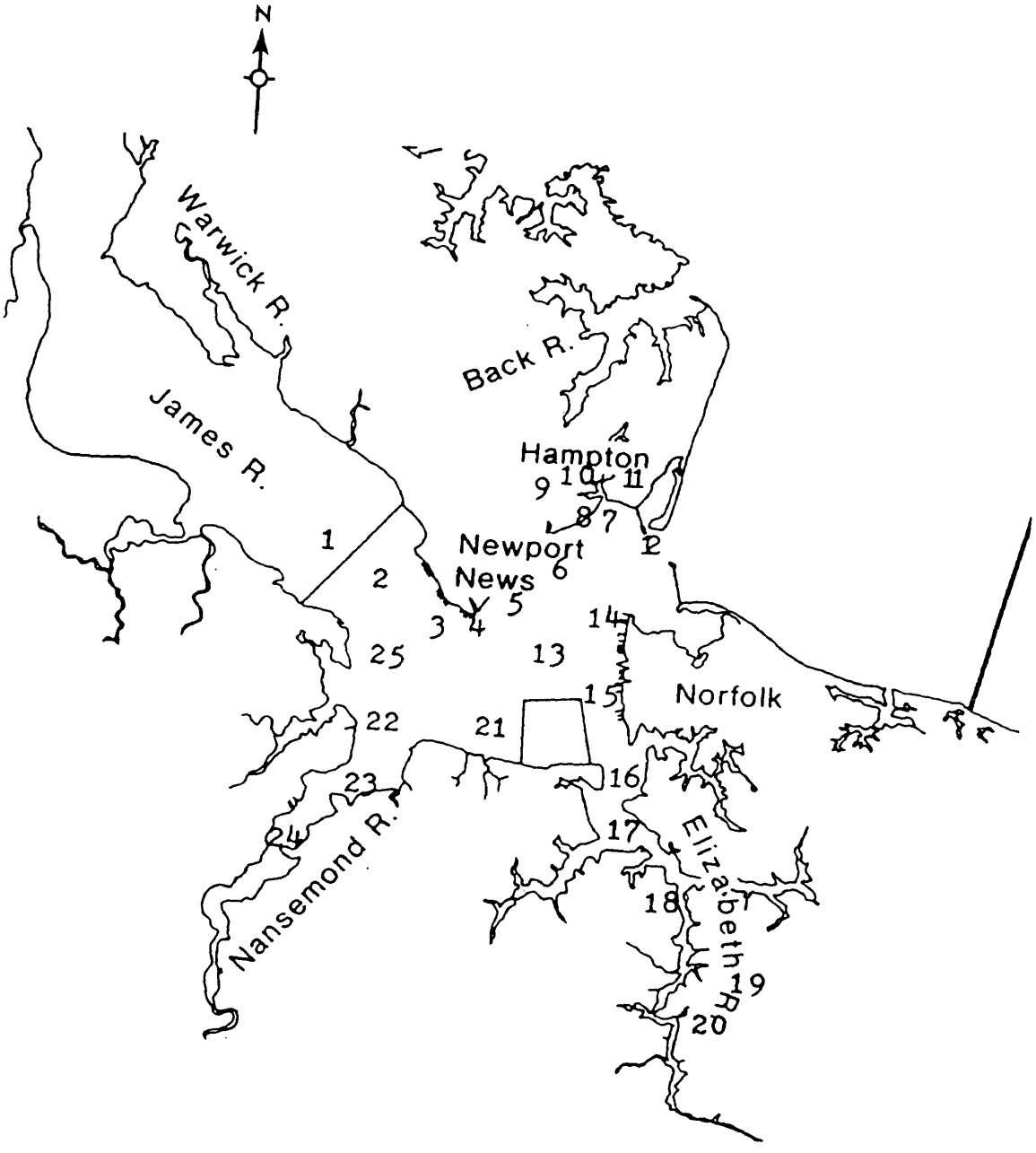
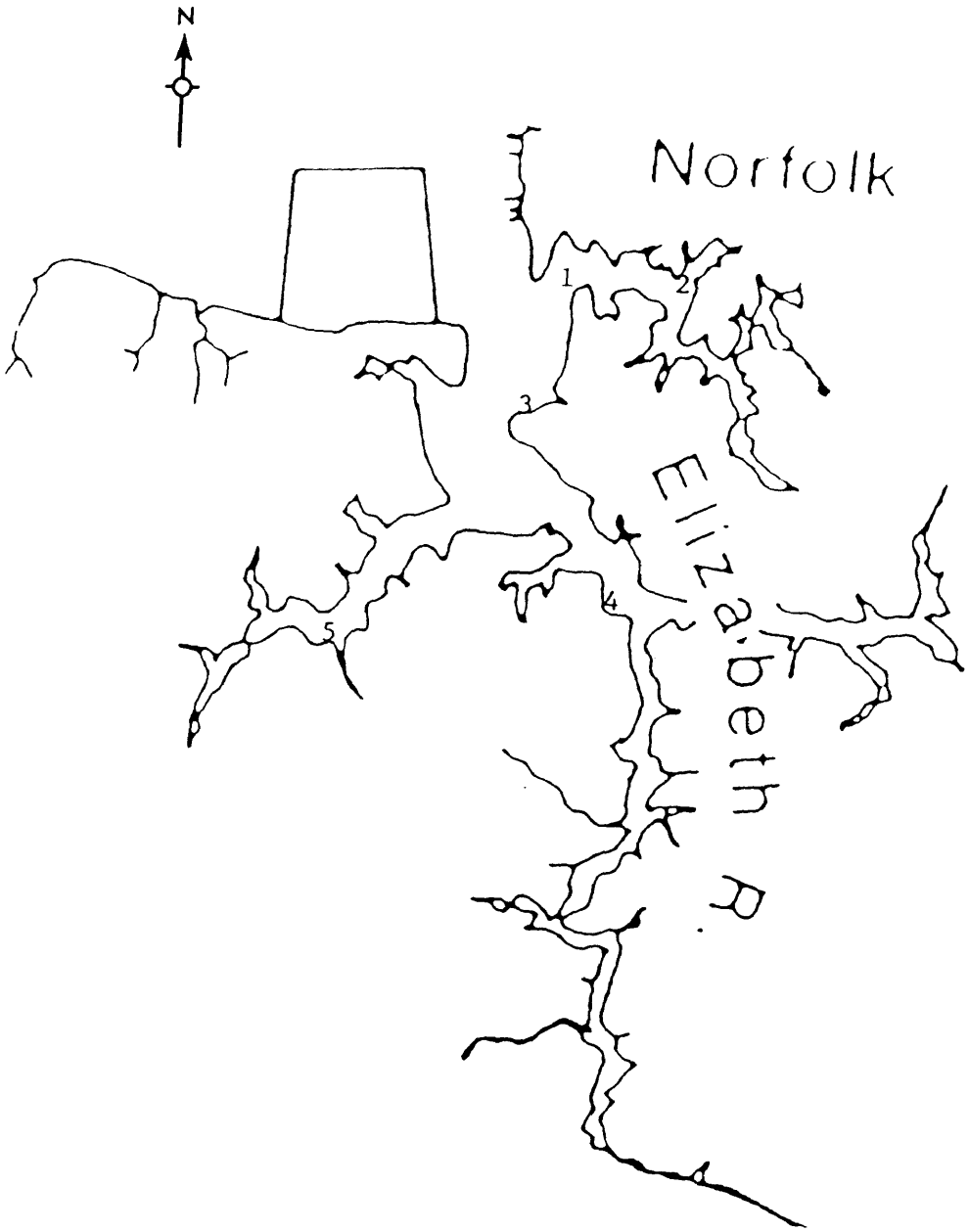

N
Warwick R.
James R.
Back R.
Hampton
Newport
News
Norfolk
Nansemond R.
Elizabeth R.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
25

Figure 7. The Elizabeth River is depicted. Both biota and sediment samples were taken. Sampling locations are shown: 1- Lafayette River, marker # 13; 2- Lafayette River, marker # 17; 3- Lambert Point; 4- Hospital Point; 5- Elizabeth River, western branch.


N
Norfolk
1
2
3
4
5
Elizabeth R.

2-3.3 Biota Collection.

Biota samples (oysters, clams, and mussels) were collected either with an oyster dredge or by hand on mud flats and in marshes. They were kept on ice while in the field and were either processed promptly after returning to the laboratory or frozen at -20 ^{o} C for later use.

2-3.4 Sample Handling.

Large debris and shells were removed from sediment samples and the contents were homogenized to insure uniformity.

Oysters, clams, and mussels were all shucked in a similar manner. The two valves were carefully pried open with an oyster knife inserted in the hinge. The animal was shaken three times, hinge down, to remove excess water trapped when shells were closed. The abductory muscles were severed, with care being taken not to damage the body and hense loose body fluids. The animal was then ground in a Virtis^{R} homogenizer, individually or in groups, and a subsample of about 5 g was set asside for moisture content analysis.

2-4 SAMPLE SIZE

Nineteen oysters were collected from the mouth of the Warwick River in the James River and analyzed individually. The mean and standard error for TBT content in oyster tissue from the single station was calculated. These results were essential in determining the sample size (n) required to assess TBT contamination in one station from a blend of "n" oysters with a set degree of precision and within a known error interval. It was calculated that oyster samples at all stations would be composed of a blend of 10 individuals. The statistical evaluation leading to this conclusion is described in the Result section. Similar study was conducted in the Rappahannock River at the Corrotoman River station where twenty oysters were collected.

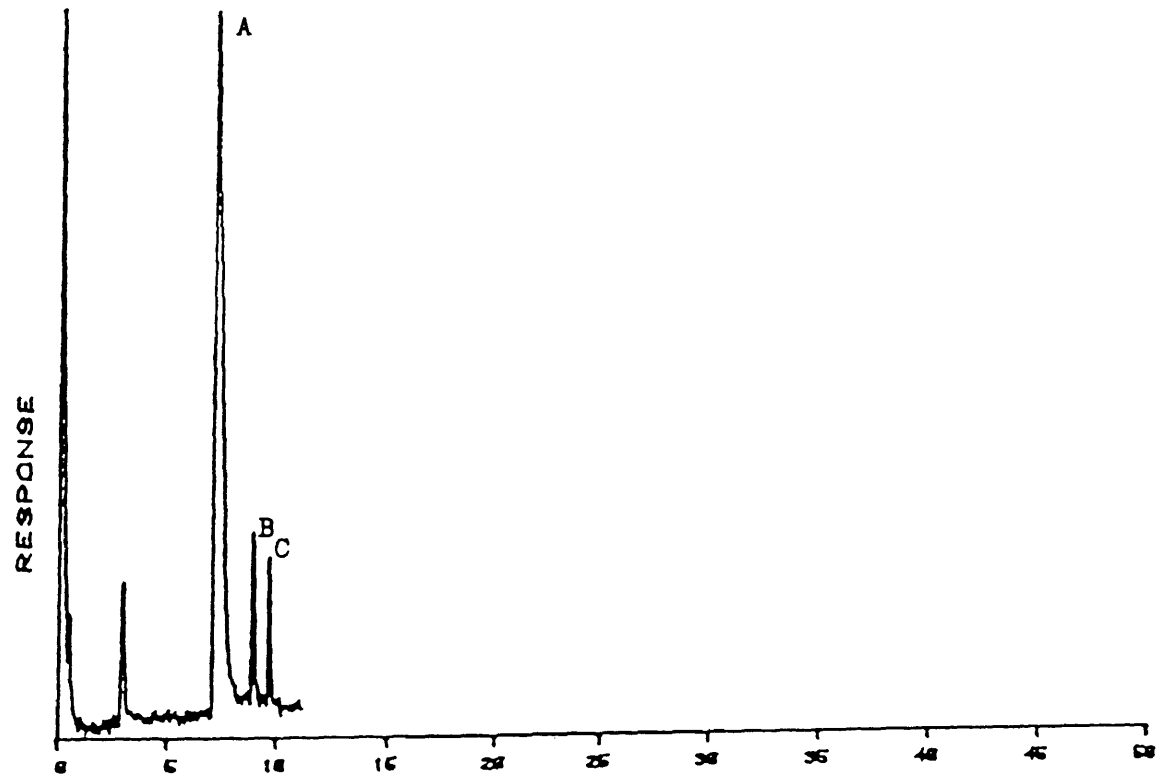
RESULTS

3-1 METHOD TESTING

3-1.1 Sediment Samples.

Extraction efficiencies for sediment samples were determined by taking five replicates from each of three series of sediments and fortifying them with 20 to 500 ug/kg TBT as tributyltin chloride. These sediments were then analyzed by the methodology described in the experimental section. A typical sediment chromatogram is shown in figure 8. Results are given in Table 3. The data show that from 92 to 106% of the TBT added was recovered. In a second experiment, a single sediment sample naturally contaminated with TBT was divided into 5 subsamples. These were extracted and analyzed for TBT. Results indicate that the mean and standard error of the mean was 50 ppb and $\pm$1.2 ppb respectively (Table 4). Five sediment samples taken at the same station were analyzed to determine the concentration variation at one station. Table 5 shows the mean and standard error of the mean at one station to be 43 ppb and $\pm$3.5 ppb respectively.

Figure 8. Typical chromatograms of butyltins in environmental samples are depicted.
Top: Typical chromatogram of an oyster sample; A. tributyltin, B. dibutyltin, C. tripentyltin (internal standard)
Bottom: Typical chromatogram of a sediment sample; A. tributyltin, B. tripentyltin.


A
B
C
RESPONSE
RETENTION TIME IN MINUTES

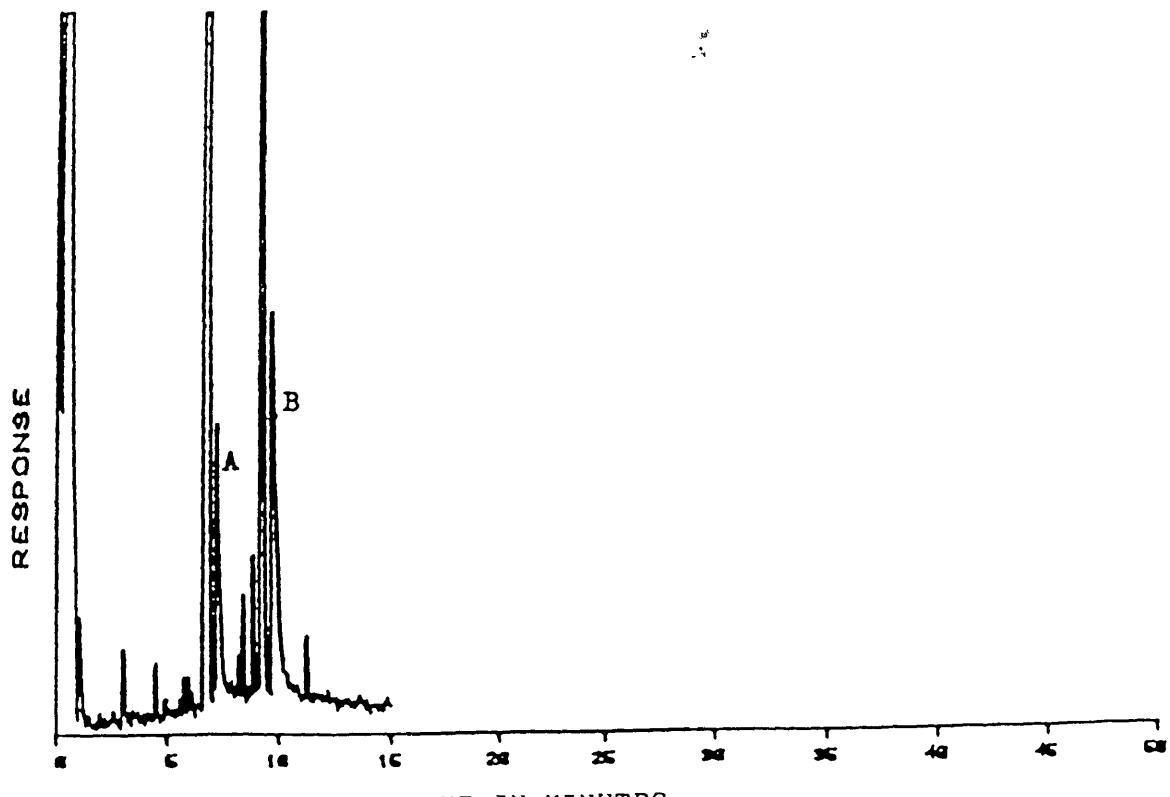

A
B
RESPONSE
RETENTION TIME IN MINUTES

Table 3.

TRIBUTYLTIN SEDIMENT FORTIFICATION EXPERIMENT

REPLICATE	SERIES 20*	100	500
1	18	92	527
2	22	88	522
3	21	113	537
4	19	88	535
5	19	80	507
$\bar{x}$ =	20	92	530
±se =	± 0.7	± 5.6	± 5.4

* Concentrations are in ug TBT+/kg dry weight (ppb).

From: Rice, Espourteille and Huggett 1987.

Table 4.

TRIBUTYLTIN VARIABILITY AMONG SUBSAMPLES
OF THE SAME SEDIMENT SAMPLE

SUBSAMPLE	TBT+*
1	47
2	53
3	48
4	51
5	52

-average TBT+ concentration : 50 ppb
-standard deviation : ± 2.6 ppb
-standard error of the mean : ± 1.2 ppb

* Concentrations are in ug TBT+/kg dry weight (ppb).

Table 5.

TRIBUTYLTIN NATURAL VARIABILITY AMONG SEDIMENTS FROM THE SAME STATION

LOCATION	TBT+*
LAFAYETTE RIVER, MARKER 13	
GRAB: a	37
GRAB: b	53
GRAB: c	41
GRAB: d	50
GRAB: e	34

-average TBT+ concentration	: 43 ppb
-standard deviation	: ± 8.2 ppb
-standard error of the mean	: ± 3.5 ppb

* Concentrations are in ug/kg dry weight (ppb).

3-1.2 Biota Samples.

TBT Extraction efficiencies for oyster tissue samples were determined by Rice et al. (1987) and ranged from 86% to 102% at concentrations of 22 ug/kg to 890 ug/kg, wet weight. A typical oyster tissue chromatogram is shown in figure 8. Simultaneous extraction efficiencies for TBT, dibutyltin (DBT) and monobutyltin (MBT) were determined at a later time using feral oysters from a pristine area. Five oysters were fortified with TBT, DBT and MBT. Recoveries ranged from 85% to 115% for a TBT concentration of 62 ug/kg, from 81% to 100% for a DBT concentration of 42 ug/kg and from 1% to 6% for a MBT concentration of 18 ug/kg, wet weight. Monobutyltin can not be recovered quantitatively with this method (Table 6).

Oysters collected from a given location displayed considerable TBT concentration variability, as seen in Tables 7 and 8. Table 7 shows the TBT concentrations of 19 oysters collected from the Warwick River mouth station in the James River. Each oyster was analyzed individually for both TBT and DBT with the method described earlier. Mean and standard error of the mean for TBT were 310 ppb and $\pm$16 ppb respectively. Mean and standard error of the mean for DBT were 24 ppb $\pm$5.8 ppb respectively. Similar results are seen in Table 8 which displays TBT values for Rappahannock River oysters. Mean and standard error of the mean for TBT and DBT were 310 ppb $\pm$18 ppb and

Table 6.

OYSTER FORTIFICATION EXPERIMENT FOR
TRIBUTYLTIN, DIBUTYLTIN, AND MONOBUTYLTIN

SAMPLE	% TBT+ RECOVERED	% DBT+ RECOVERED	% MBT+ RECOVERED
1	105	88	6
2	92	81	6
3	97	86	<1
4	115	100	<1
5	85	81	6
$\bar{x}$	98.8	87.2	4
$\pm$se	$\pm$5.2	$\pm$3.5	$\pm$1.2

Homogenized oyster tissue was fortified with a TBT+, DBT+ and MBT+ solution. Concentrations of the species were as follow: TBT+ 62 ppb, DBT+ 42 ppb, and MBT+ 18 ppb.

Table 7.

TRIBUTYLTIN NATURAL VARIABILITY IN OYSTERS* FROM THE JAMES RIVER

LOCATION	TBT+	DBT+
JAMES RIVER**, Warwick river mouth		
OYSTER # 1	270	17
OYSTER # 2	260	27
OYSTER # 3	190	n.d.***
OYSTER # 4	460	29
OYSTER # 5	250	28
OYSTER # 6	310	n.d.
OYSTER # 7	350	97
OYSTER # 8	230	28
OYSTER # 9	330	48
OYSTER # 10	320	75
OYSTER # 11	370	17
OYSTER # 12	400	15
OYSTER # 13	430	n.d.
OYSTER # 14	280	17
OYSTER # 15	240	6.9
OYSTER # 16	340	17
OYSTER # 17	330	21
OYSTER # 18	290	n.d.
OYSTER # 19	290	19
Average value per oyster	310	24
Standard deviation	70	25
Standard error of the mean	16	5.8

* Concentrations are in ug/kg dry weight (ppb).

** All oysters were collected on 22 June 1987 from the James River, at the Warwick River mouth.

*** n.d.: Not detectable at 10 ppb dry weight.

Table 8.

TRIBUTYLTIN NATURAL VARIABILITY IN OYSTERS*
FROM THE RAPPAHANNOCK RIVER

LOCATION	TBT+	DBT+
RAPPAHANNOCK RIVER**, Corrotoman river station		
OYSTER # 1	270	27
OYSTER # 2	200	21
OYSTER # 3	250	19
OYSTER # 4	350	36
OYSTER # 5	310	24
OYSTER # 6	350	37
OYSTER # 7	310	n.d.***
OYSTER # 8	230	n.d.
OYSTER # 9	220	24
OYSTER # 10	240	32
OYSTER # 11	280	28
OYSTER # 12	250	38
OYSTER # 13	350	n.d.
OYSTER # 14	550	n.d.
OYSTER # 15	370	n.d.
OYSTER # 16	350	27
OYSTER # 17	450	n.d.
OYSTER # 18	300	n.d.
OYSTER # 19	310	21
OYSTER # 20	320	n.d.
Average value per oyster	310	17
Standard deviation	82	15
Standard error of the mean	18	3.3

* Concentrations in ug/kg dry weight (ppb).

** All oysters were collected on 29 June 1987 from the Rappahannock River, Corrotoman River station.

*** n.d.: Not detectable at 10 ppb dry weight.

17 ppb $\pm$3.3 ppb respectively, which roughly approximates that of James River oysters.

3-1.3 Statistics.

Results from Tables 7 and 8 were used to determine the sample size (n) required to assess TBT contamination at a station from a blend of "n" oysters. According to Zar (1984) the following equation allows for the determination of the sample size needed to estimate the population mean with the desired precision:

$$N = (S^2 \, T^2_{a(2),(n-1)} \, F_{b(1),(n-1,v)})/d^2$$

where N is the sample size, S^2 is the variance of the 19 oysters sampled, which is an estimate of the population variance (Table 7); $T_{a(2),(n-1)}$ is the two tailed critical value of Student`s T, "a" being the confidence interval with n-1 degrees of freedom; $F_{b(1),(n-1,v)}$ is the one tail critical value for the F distribution, "b" being the assurance that the confidence interval will be no larger than specified with n-1 and "v" degrees of freedom; "d" is the half width of the desired confidence interval and it was set to equal the standard deviation, which for Table 7 is equal to 70 ppb. It was decided to set the confidence level (a) at 95%. The power of the test was set at 90% which specifies a 90 % probability that the confidence interval will be no larger that specified (b). The sample size (n) then equals 10. Therefore oyster samples at all stations were composed of a blend of 10 individuals.

3-2 TRIBUTYLTIN IN SEDIMENTS

3-2.1 Hampton Roads; July and August 1986.

All values are reported as dry weight. In all cases, detection limit is defined as two times the signal to noise ratio. Highest sediment TBT concentrations were found in Hampton Roads during the summer of 1986 (figure 6). Stations 8 through 11 (Table 9) reveal very high TBT contamination (> 100 ppb), station 9 being the highest found in both sampling trips (1000 ppb and 4000 ppb). This sampling site was close to dry dock facilities and it is possible that TBT paint chips were present. These sample sites (8-11) are located inside the Hampton Roads marina where hundreds of boats are moored year round. Elizabeth River samples (stations 15-20) ranged from moderate TBT concentration (between 10 ppb and 50 ppb) at stations 15, 17 and 20 to very high TBT concentration (> 100 ppb) at stations 16, 18 and 19 during the month of July. In August, stations 15 and 17 had moderate TBT concentrations, stations 16 and 20 had high TBT concentrations and stations 18 and 19 had very high TBT concentrations. Samples from the Nansemond River mouth (stations 22-25) registered moderate to low (< 10 ppb) TBT values in July; station 24 contained 11ppb TBT and station 22 was not detectable at 1 ppb. In August all four

Table 9.

TRIBUTYLTIN CONCENTRATIONS IN
HAMPTON ROADS AND ELIZABETH RIVER SEDIMENTS

STATION	TBT+ ug/kg dry weight	
	JULY 1986	AUGUST 1986
1	26	27
2	12	16
3	41	40
4	5.6	4.0
5	3.4	5.0
6	7.1	5.9
7	51	98
8	120	190
9	1000	4000
10	190	290
11	440	260
12	34	31
13	5.9	51
14	29	7.3
15	24	34
16	590	78
17	42	47
18	230	100
19	130	110
20	35	90
21	21	14
22	n.d.*	4.9
23	10	6.6
24	11	8.9
25	5.7	9.8

* Not detectable at 1 ppb dry weight.

stations had low TBT concentrations. Low TBT concentrations were also found in stations 4, 5 and 6 during both sampling trips.

3-2.2 Elizabeth and Lafayette Rivers.

Concentrations as high as 300 ppb were encountered in Elizabeth River sediments (figure 7) at the Lambert Point station in the summer of 1987. Other results fell generally between 30 and 60 ppb. The lowest value was in the Elizabeth River Western Branch at 34 ppb (Table 10).

3-2.3 James River.

Sediment samples from the James River (figure 2) contained less TBT than their Elizabeth River counterparts. The Jail Island station had the lowest concentration with 2.0 ppb. The sediments from Nansemond Ridge contained 36 ppb (Table 11) and were the highest for this river.

3-2.4 Rappahannock River.

All Rappahannock River sediments (figure 3) were below detection limit (Table 12), which in this case ranged from 1 to 14 ppb (dry weight). As stated previously, the detection limit is defined as two times the signal to noise ratio. The detection limit was unusually high for some of these sediments. The samples consisted of anoxic sediments which contained compounds which interfered during the gas chromatography analyses.

Table 10.

TRIBUTYLTIN CONCENTRATIONS IN
ELIZABETH AND LAFAYETTE RIVERS SEDIMENTS

LOCATION	TBT+
ELIZABETH RIVER	
WESTERN BRANCH	34*
HOSPITAL POINT	200
LAMBERT POINT	300
LAFAYETTE RIVER	
MARKER #13	43
MARKER #17	58

-All samples were collected on 20 July 1987.

*Concentrations are in ugTBT+/kg dry weight (ppb).

Table 11.

TRIBUTYLTIN CONCENTRATIONS IN
JAMES RIVER SEDIMENTS

LOCATION	TBT+
WHITE SHOAL	17*
THOMAS ROCK	30
PAGAN RIVER MOUTH	25
NASEWAY SHOAL	9.0
BROWN SHOAL	20
POINT OF SHOAL	6.3
HORSE HEAD	11
DEEP WATER SHOAL	7.2
WARWICK RIVER MOUTH	8.5
JAIL IS., NELLS CR.	2.0
WRECK SHOAL	7.3
NANSEMOND RIDGE	36

-All samples were collected on 22 June 1987.

*Concentrations are in ug TBT+/kg dry weight (ppb).

Table 12.

TRIBUTYLTIN CONCENTRATIONS IN
RAPPAHANNOCK RIVER SEDIMENTS

LOCATION	TBT+*	DETECTION LIMIT
CORROTOMAN RIVER	n.d.**	9.0
PARROTS ISLAND	n.d.	7.5
PUNCH BOWL	n.d.	4.5
HOG HOUSE BAR	n.d.	14
BELLE ISLAND	n.d.	1.0
MORATTICO BAR	n.d.	8.0
BOWLERS ROCK	n.d.	7.0
SMOKEY POINT	n.d.	14
MONASKON BLUFF	n.d.	1.5

-All samples were collected on 29 June 1987.

* Concentrations are in ug TBT+/kg dry weight (ppb).

** Not detectable at -ppb, dry weight.

3-2.5 Great Wicomico River.

TBT in the Great Wicomico River sediments (figure 4) ranged from a high of 63 ppb at Glebe Point to less than the detection limit of 14 ppb at Rogue Point (Table 13). These sediments, like those from the Rappahannock River, contained interfering compounds.

3-2.6 Eastern Shore.

Sediment samples from the bay side of the Eastern Shore (figure 5) at Messongo Creek, Plantation Creek and Onancock Creek bay side were not detectable at 1 ppb, dry weight (Table 14). Sediments from other locations sampled ranged from a low of 1.4 ppb at Occanannock creek to a high of 93 ppb at Cherrystone Inlet, for those stations above detection limit.

Samples from the ocean side of the Eastern Shore (figure 5) were consistently low in TBT. The detection limit was again 1 ppb and a third of the stations sampled contained less than this limit (Table 15). TBT concentrations spanned a range from 1.3 ppb in Chicoteague bay to 5.8 at Folly creek.

Table 13.

TRIBUTYLTIN CONCENTRATIONS IN
GREAT WICOMICO RIVER SEDIMENTS

LOCATION	TBT+
HAYNIE POINT	20*
BUSSEL POINT	18
GLEBE POINT	63
FLEET POINT	22
ROGUE POINT	n.d.**

-All samples were collected on 11 August 1987.

* Concentrations are in ug TBT+/kg dry weight (ppb).

** Not detectable at 14 ppb dry weight.

Table 14.

TRIBUTYLTIN CONCENTRATIONS IN
EASTERN SHORE SEDIMENTS (bay side)

LOCATION	TBT+
MESSONGO CREEK, rt 788	n.d.*
HUNGARS CREEK, rt 657	40**
HUNGARS WHARF, rt 666	4.1
CHERRYSTONE INLET, rt 663	93
ONANCOCK CREEK (bay side), rt 767	n.d.
ONANCOCK CREEK (marina), rt 767	5.2
HACKSNECK, rt 759	23
OCCAHANNOCK CREEK, rt 611	1.4
NASSAWADOX CREEK, rt 713	20
PLANTATION CREEK, rt 644	n.d.

-All samples were collected between 25 August 1987 and 27 August 1987.

* Non detectable at 10 ppb dry weight.

** Concentrations are in ug TBT+/kg dry weight (ppb).

Table 15.

TRIBUTYLTIN CONCENTRATIONS IN
EASTERN SHORE SEDIMENTS (ocean side)

LOCATION	TBT+
CHINCOTEAGUE BAY, (1)	1.3*
CHINCOTEAGUE BAY, (2)	n.d.**
MACHIPONGO RIVER	n.d.
BOGUES BAY	3.2
WILLIS WHARF	n.d.
FOLLY CREEK	5.8
WACHAPREAGUE	3.6
METOMPKIN BAY (near shore)	1.8
METOMPKIN BAY (barrier Is)	n.d.
COBB BAY	n.d.
OYSTER (town)	1.6

-All samples were collected between 25 August 1987 and 27 August 1987.

* Concentrations are in ug TBT+/kg dry weight.

** Not detectable at 1 ppb dry weight.

3-3 TRIBUTYLTIN IN BIOTA

3-3.1 Elizabeth and Lafayette Rivers.

All biota TBT values are listed as dry weight. As with sediment samples the detection limit is defined as twice the signal to noise ratio. Biota concentrations in the Elizabeth River (figure 7) are listed in Table 16. The Hospital Point station contained 5600 ppb and values from all other stations, from both the Elizabeth and Lafayette Rivers were greater than 1000 ppb. All samples contained DBT.

3-3.2 James River.

The survey of the James River (figure 2) revealed that oysters contained TBT levels as high as 1200 ppb (Horse Head and Brown Shoal). The Jail Island station had the lowest value with 290 ppb. Only samples from three stations revealed DBT (Table 17).

3-3.3 Rappahannock River.

Oyster samples from the Rappahannock River (figure 3) ranged from a high of 1100 ppb at Hog House Bar, which is located near Urbanna's marina, to a low of 240 ppb at Bowlers Rock. More than half the oysters sampled were found to have DBT (Table 18).

3-3.4 Great Wicomico River.

On the average, the Great Wicomico River (figure 4) biota samples contained more TBT than their Rappahannock River counterparts (Table 19). Glebe Point has the highest concentration with 1400 ppb while Bussel Point has the lowest with 520 ppb. All oyster samples contained DBT in amounts generally one order of magnitude below TBT concentrations.

3-3.5 Eastern Shore.

On the bay side of the Eastern Shore of Virginia (figure 5), TBT concentrations in biota varied greatly from 2000 ppb at Onancock creek marina to 34 ppb at Occonannock creek. Intermediate values of 100 to 500 ppb were common. DBT concentrations followed closely TBT concentration trends, but were generally one order of magnitude lower (Table 20).

On the ocean side of the Eastern Shore of Virginia (figure 5) only two biota samples exceeded 100 ppb; both were from the Wachapreague station. Oysters contained 180 ppb while clams reached 290 ppb. Other samples (Table 21) ranged between 20 and 90 ppb, with the exception of the Chincoteague Bay (rt 709) sample which was 6.9 ppb.

Table 16.

TRIBUTYLTIN CONCENTRATIONS IN
ELIZABETH AND LAFAYETTE RIVERS BIOTA

LOCATION	AVE. TISSUE WEIGHT (g)	AVE. SHELL LENGHT (cm)	TBT+	DBT+
ELIZABETH RIVER:				
WESTERN BRANCH	13.4±4.40	7.88±4.55	1400*	130
HOSPITAL POINT	8.26±2.11	6.28±0.78	5600	500
HOSPITAL POINT**	21.0	6.0	3300	390
LAMBERT POINT	9.94±2.37	6.45±0.71	1300	110
LAFAYETTE RIVER				
MARKER # 13	9.32±4.42	6.43±1.41	2300	280
MARKER # 17	21.1±8.16	8.80±1.30	3400	320

-All samples were collected on 20 July 1987. Samples are a blend of 10 oysters, Crassostrea virginica, unless otherwise specified. For each station the 10 individuals were measured and their tissue weighed; both means are reported above with their standard errors.

* Concentrations are in ug TBT+/kg dry weight (ppb).

** Hard clam, Merceneria merceneria; sample size is 1.

Table 17.

TRIBUTYLTIN CONCENTRATIONS IN
JAMES RIVER OYSTERS

LOCATION	TBT+	DBT+
WARWICK RIVER MOUTH	300*	24
WHITE SHOAL	950	n.d.**
THOMAS ROCK	760	n.d.
PAGAN RIVER	580	n.d.
NASEWAY SHOAL	620	n.d.
BROWN SHOAL	1200	87
POINT OF SHOAL	710	70
HORSE HEAD	1300	n.d.
DEEP WATER SHOAL	670	n.d.
JAIL IS., NELL'S CREEK	290	n.d.
WRECK SHOAL	690	n.d.
NANSEMOND RIDGE	500	n.d.
NANSEMOND RIDGE***	110	77

-All samples were collected on 22 June 1987. Samples are a blend of 10 oysters, Crassostrea virginica, unless otherwise specified. For each station the 10 individuals were measured and their tissue weighed; both means are reported above with their standard errors.

*Concentrations are in ug/kg dry weight (ppb).

** Not detectable at 20 ppb dry weight.

*** pea crab sample, Pinnotheres ostreum; sample size is 6.

Table 18.

TRIBUTYLTIN CONCENTRATIONS IN
RAPPAHANNOCK RIVER OYSTERS

LOCATION	AVE. TISSUE WEIGHT (g)	AVE. SHELL LENGHT (cm)	TBT+	DBT+
CORROTOMAN RIVER	n.a.*	n.a.	310**	17
PARROT ISLAND	17.3±8.60	10.5±3.52	530	n.d.***
HOG HOUSE BAR	20.1±7.78	8.85±1.32	1100	140
PUNCH BOWL	21.1±6.23	9.13±1.02	470	52
BELLE ISLAND	27.3±12.2	10.3±1.29	380	n.d.
MORATTICO	26.2±3.25	9.85±0.90	360	46
BOWLERS ROCK	26.9±7.91	9.48±1.43	240	n.d.
SMOKEY POINT	21.1±5.64	9.10±1.20	540	70
MONASKON BLUFF	21.9±7.24	9.45±0.89	370	48

-All samples were collected on 29 June 1987. Samples are a blend of 10 oysters, Crassostrea virginica, except for the Corrotoman River station where a blend of 20 oysters was used. For each station the 10 individuals were measured and their tissue weighed; both means are reported above with their standard errors.

* Not available.

** Concentrations are in ug TBT+/kg dry weight (ppb).

*** Not detectable at 40 ppb dry weight (ppb).

Table 19.

TRIBUTYLTIN CONCENTRATIONS IN
GREAT WICOMICO RIVER OYSTERS

LOCATION	AVE. TISSUE WEIGHT (g)	AVE. SHELL LENGHT (cm)	TBT+	DBT+
HAYNIE POINT	9.76±5.27	7.35±1.58	610*	110
BUSSEL POINT	7.29±2.60	6.55±0.93	520	67
GLEBE POINT	15.9±2.33	8.08±0.96	1400	140
FLEET POINT	7.83±5.66	6.83±1.91	600	73
ROGUE POINT	13.0±5.34	7.95±1.12	930	95

-All samples were collected on 11 August 1987. Samples are a blend of 10 oysters, Crassostrea virginica, in all cases. For each station the 10 individuals were measured and their tissue weighed; both means are reported above with their standard errors.

* Concentrations are in ug TBT+/kg dry weight (ppb).

3-4 TRIBUTYLTIN CONFIRMATION BY GC MASS SPECTROMETRY

Presence of TBT in environmental samples was confirmed by gas chromatography mass spectrometry. Four biota samples containing high TBT concentrations were analyzed. These samples came from Hog House Bar in the Rappahannock River, from Hospital Point in the Elizabeth River, from Horse Head in the James River and from Rogue Point in the Great Wicomico River. Figure 9 depicts a typical mass spectrum of TBT in an environmental biota sample.

Table 20.

TRIBUTYLTIN CONCENTRATIONS IN
EASTERN SHORE BIOTA (Bay Side)

LOCATION	AVE.TISSUE WEIGHT (g)	AVE. SHELL LENGHT(cm)	TBT+	DBT+
MESSONGO CREEK*	4.40±4.44	5.08±2.18	41**	6.0
PLANTATION CREEK	7.19±6.90	6.48±2.11	49	6.4
HUNGARS CREEK	10.3±4.64	7.50±1.31	150	27
HUNGARS WHARF	6.83±5.97	7.03±1.77	1900	130
OCCONANNOCK CR	3.86±1.50	5.18±0.55	43	8.6
OCCONANNOCK CR***	7.80±3.91	6.48±0.84	34	nd****
HACKSNECK	9.45±5.21	7.10±1.76	200	28
ONANCOCK CR, BAY	12.4±8.95	7.05±1.89	110	20
ONANCOCK CR, MARINA	8.15±4.18	6.15±1.18	2000	340
CHERRYSTONE INLET	6.67±3.84	6.43±1.42	510	50
NASSAWADOX CREEK	4.80±2.42	5.73±1.39	320	27

-All samples were collected between 25 August 1987 and 27 August 1987. Samples are a blend of 10 oysters, Crassostrea virginica, unless otherwise specified. For each station the 10 individuals were measured and their tissue weighed; both means are reported above with their standard errors.

* Sample size is 6.

** Concentrations are in ug TBT+/kg dry weight (ppb).

*** Mussel sample, Modiolus demissus.

**** Not detectable at 1 ppb dry weight.

Table 21.

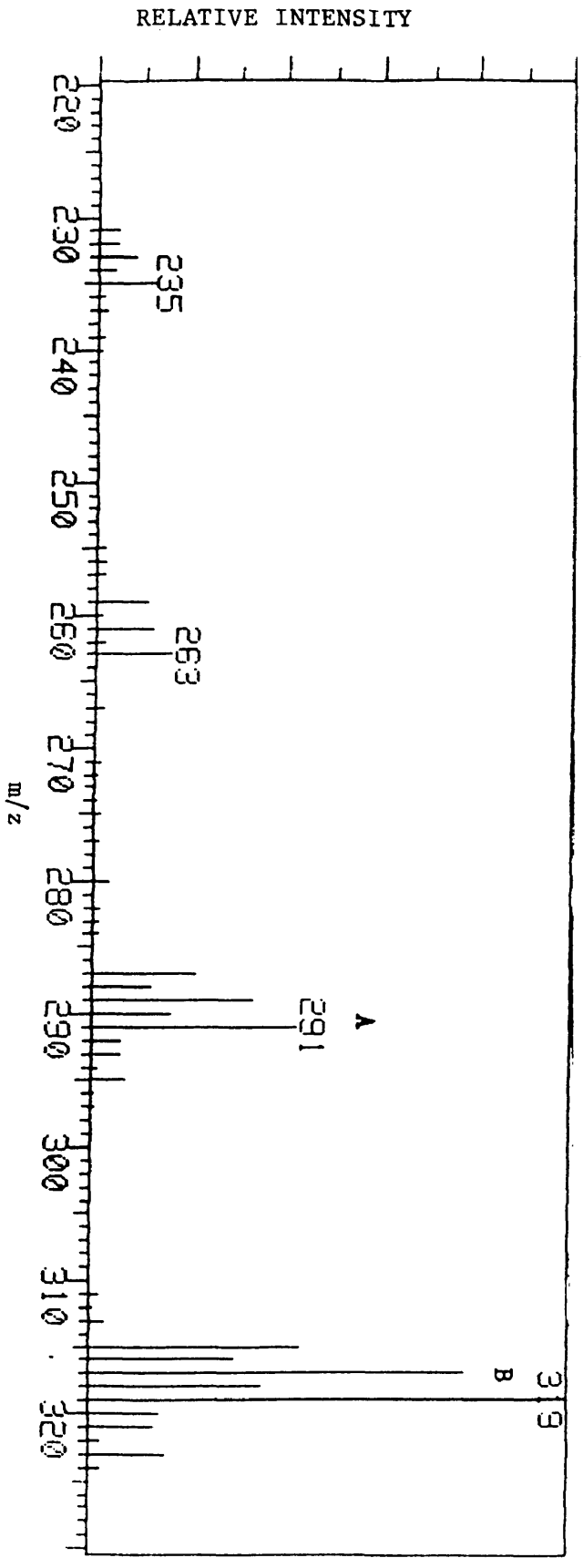
TRIBUTYLTIN CONCENTRATIONS IN
EASTERN SHORE BIOTA (Ocean Side)

LOCATION	AVE. TISSUE WEIGHT (g)	AVE. SHELL LENGHT(cm)	TBT+	DBT+
CHINCOTEAGUE BAY(1)	7.48±4.00	7.01±1.43	24[a]	6.2
CHINCOTEAGUE BAY(2)	4.59±1.65	5.50±0.62	6.9	1.6
MACHIPONGO RIVER	6.61±3.75	8.13±2.87	50	9.0
BOGUE BAY	7.76±2.92	6.58±1.14	37	8.2
WILLIS WHARF	8.43±2.92	9.48±1.73	43	5.3
FOLLY CREEK	5.54±1.31	5.73±0.92	93	13
FOLLY CREEK[b]	-----	-----	34	8.1
FOLLY CREEK[d]	8.39±5.00	7.25±1.65	36	5.5
OYSTER (town)	6.85±3.70	6.93±1.78	29	3.7
COBB BAY	8.43±4.49	7.28±1.59	21	nd[e]
WACHAPREAGE	7.78±2.67	8.65±1.66	180	18
WACHAPREAGE[f]	43.2±12.5	7.96±0.74	290	36
METOMPKIN BAY shore	6.51±3.58	7.28±1.36	29	5.2
METOMPKIN BAY inlet[g]	30.3±10.6	6.93±0.76	51	9.2
METOMPKIN BAY inlet[h]	7.79	4.0	20	18

All samples were collected between 25 August 1987 and 27 August 1987. Samples are a blend of 10 oysters, Crassostrea virginica, unless otherwise specified. For each station the 10 individuals were measured and their tissue weighed; both means are reported above with their standard errors.

[a] Concentrations are in ug TBT+/kg dry weight.
[b] Mud snail sample, Illynassa obsoleta.
[d] Mussel sample, Modiolus demissus.
[e] Not detectable at 0.5 ppb dry weight.
[f] Clam sample, Merceneria merceneria.
[g] Clam sample, M. merceneria.
[h] Blood clam sample, Anadara ovalis; sample size is 1.

Figure 9. A typical mass spectrum of tributyltin in an environmental sample is depicted:
A. $[(C_4H_9)_3Sn^+]$
B. $[(C_4H_9)_2(C_6H_{13})Sn^+]$
(Great Wicomico River, Rogue Point station)


RELATIVE INTENSITY
m/z
220
230
235
240
250
260
263
270
280
290
291
A
300
310
315
320
B

DISCUSSION

4-1 HEAVILY POLLUTED AREAS

4-1.1 Hampton Roads and Elizabeth River.

Of all the areas surveyed, Hampton Roads (1986 samples) and the Elizabeth River (1987 samples) had the highest degree of TBT pollution. Commercial, naval and recreational boating traffic is extensive in the area and results found in tables 10 and 16 are apparently a consequence of this. The most polluted sediment encountered came from Hampton marina (station 9 in Table 9). It contained 4000 ppb TBT dry weight. This station is located in an area surrounded by marinas in which a total of several hundred boats are moored year round.

Generally, stations showing high sediment levels had higher levels in biota, although no direct relationship was evident.

The Elizabeth River Hospital point station contained oysters with the highest TBT concentration at 5600 ppb TBT. All Elizabeth River stations have high levels of the contaminant. The Elizabeth River is relatively narrow and deep and it supports intense boat traffic. This river does not receive much fresh water inflow.

4-2 MODERATELY POLLUTED AREAS

4-1.2 Great Wicomico River.

The Great Wicomico River carries a high TBT pollution burden in biota with a river average value between 500 and 1000 ppb. Pollution burden in sediments is moderate, with a river average value between 10 and 50 ppb (Tables 13 & 19). The net flow of this river is low which does not promote pollutant removal from the system. Small fishing and pleasure crafts use the river. A boat yard for small vessels is found between the Glebe Point and Haynie Point stations, which could provide a TBT source. It should be noted that these two stations have the highest TBT concentration in biota and nearly the highest concentrations in sediments.

4-2.2 James River.

The James River is responsible for an important oyster fishery. Pollution load for sediments and biota is moderate and high, respectively. Higher TBT concentrations in sediments are found close to the James River bridge (Table 11) and oysters from two stations exceed 1000 ppm TBT (Table 17). Commercial and pleasure boats are numerous in the river. The U.S. Navy uses the river extensively but the majority of their vessels do not have TBT paint. The James River should benefit from the ban on TBT enacted in March 1987. The net fresh water

flow to this river exceeds 10000 cubic feet per second (yearly average)and small crafts should be devoid of TBT paint in one or two years. Even though some large cargo ships and tankers (likely to have TBT paint) will continue to use the river, a decrease in TBT pollution is likely.

At the Nansemond Ridge station TBT values for both oysters and pea crabs (Pinnotheres ostreum), found in the oysters, were obtained. Values for the pea crab were much lower that those of oysters at that station. Crustaceans, unlike bivalves, are capable of metabolizing TBT to a certain extent. This could explain the lower TBT concentration in the crab sample relative to oysters. The crab sample had a TBT-DBT ratio of 1:0.75 which was much higher than the typical bivalve ratio of 1:0.1 found in this study. This supports the presence of TBT metabolism in crusteceans.

4-2.3 Rappahannock River.

Oysters from the Rappahannock were the largest sampled. Mean tissue weight and mean shell lengh for Belle Isle station exceeded 27 g (wet weight) and 10 cm per oyster, respectively (Table 18). Pollution burden in sediments and biota is low (< 10 ppb) and moderate, respectively. With the exception of animals from Hog House bar all Rappahannock River biota samples were relatively close in TBT concentration. The reason why TBT concentration in oyster tissue from Hog House bar exceeded

by two and half times that of the rest of river is likely due to the fact that this site is located just outside and slightly downstream from a marina of the city of Urbanna. It is the largest marina on the Rappahannock River.

4-2.4 Bay Side of the Eastern Shore.

The bay side of the Eastern Shore of Virginia displayed a great range of contamination due to variation in boating activity. Areas with heavy boating activity such as Hungars Wharf and Onancock Creek Marina contain very high (> 1000 ppb) TBT concentrations in biota. An average value for TBT concentration in biota indicates a moderate pollution load (between 100 and 500 ppb). Sediment values vary greatly between 93 ppb at the Cherrystone Inlet station to less than 1 ppb at more remote sites. An average value for TBT concentration in sediments suggests a moderate pollution burden (between 10 and 50 ppb) (Tables 14 & 20).

To futher exemplify the importance of boats in contaminating the marine environment with TBT, one can compare the two Onancock creek stations. In the open bay station, which is devoid of boats, TBT concentration in biota and sediments are 110 ppb and less than 1 ppb respectively. Further down the creek, in an enclosed area containing a marina with moored boats, TBT concentrations in biota and sediments reached 2000 ppb and 5.2 ppb,

respectively. Boats would seem to have a direct influence on environmental TBT concentrations. Stations with reduced boating activities or where strong currents prevail have TBT concentration in biota less than 50 ppb and sediment concentrations 1 ppb or less.

4-3 MINIMALLY POLLUTED AREAS

4-3.1 Ocean Side of the Eastern Shore.

Results from the ocean side of the Eastern Shores contained the lowest TBT concentration overall in both sediments (< 10 ppb) and biota (< 100 ppb). Sediments which did not contain TBT above detection levels came from remote stations with little boating activities or stations which have direct access to the open ocean. While sediment samples from some ocean side Eastern Shore locations were found to be below detection limits, TBT was found in all the tissue samples (Table 21). The two Wachapreague samples were an exception among the biota samples as they contained more than 100 ppb of TBT. They were collected next to a marina where 70 to 80 boats were moored. Among the stations where high TBT levels were found, the Folly creek station is adjacent to a boat ramp and the creek itself supports heavy boating activity. Both Magothy Bay and Chincoteague Bay (rt 709), which have relatively low TBT contamination, are large shallow bays with few boats.

An interesting anomaly can be found in the TBT

concentration of the blood clam (Anadara ovalis) collected at the Metompkin Bay inlet station. TBT and DBT values are nearly the same which is different from all the bivalves sampled so far, M. merceneria and C. virginica. The blood clam is apparently capable to metabolize TBT since its TBT content is less than half and its DBT content is twice that of M. merceneria from the same station. A. ovalis possesses hemoglobin, which sets it apart from M merceneria from the point of view of evolution and suggests physiological differences.

4-4 DETOXIFICATION OF SELECTED SITES.

A potentially important aspect of this study, when combined with other research, is that it may enable a prediction of the time required for sediments to be freed of TBT. As we know, bottom sediments are contaminated with TBT. These sediments can act as a source of TBT, releasing it to the water. Distribution coefficients between sediments and water typically range from 1000 to 10000 (Unger, personnal communication). A survey of the litterature revealed that the governing factor for removal of TBT from a marine system, such as the Chesapeake Bay, was TBT degradation in sediments. In the sediments, TBT is debutylated to DBT. A half life of 162 days for TBT in sediments has been reported by Stang (1986). Table 22

Table 22.

TIME TABLE FOR
THE NATURAL DETOXIFICATION OF SELECTED SITES

LOCATION	EXPECTED RESIDUAL TBT+ LEVEL IN SEDIMENTS			
	1.0 ppb	0.5 ppb	0.1 ppb	0.001 ppb
JAMES RIVER	1.59-0.31*	1.90-0.62	2.61-1.33	4.66-3.37
(average)	1.20**	1.51	2.22	4.27
RAPPAHANNOCK RIVER	0.86-0.00	1.17-0.00	1.89-0.71	3.93-2.76
(average)	0.58	0.89	1.90	3.65
GREAT WICOMICO RIVER	1.84-0.86	2.15-1.17	2.86-1.89	4.90-3.93
(average)	1.45	1.75	2.47	4.90
EASTERN SHORE bay side	2.01-0.00	2.32-0.00	3.03-0.71	5.08-2.76
(average)	1.31	1.61	2.33	4.37
EASTERN SHORE ocean side	0.78-0.00	1.09-0.00	1.80-0.71	3.85-2.76
(average)	0.21	0.52	1.23	3.27

Values in the above table depict the time (in years) needed for TBT+ concentration in sediments to reach the TBT+ levels listed at the top of each columns. The average time reported was calculated using the current TBT+ pollution averages for each river (for the Rappahannock, which sediments were below detection limit, TBT+ values of half the detection limit at each station were assumed). The values listed above the average values (high-low) were calculated from the highest and lowest values found in each areas. These values were calculated from the degradation rate of TBT+ in sediments (TBT half life in sediments is 162 days (Stang1986)), with the following equation: $N_t = N_o e^{-rt}$, where N_o is the initial TBT concentration, r is the degration rate, t is the elapsed time and N_t is the TBT concentration at time t.

* value range.
** value average.

various time estimates for TBT detoxification in the areas sampled. These values were calculated with the following equation: $N_t = N_o e^{-rt}$, where N_t is the initial TBT concentration (concentration at time zero), r is the degradation rate, t is the elapsed time and N_t is the final TBT concentration (concentation at time t). To reach a 1 ppb TBT concentration level in sediments from these areas one to two years may be required, while five or more years may be necessary to decrease pollution levels to 0.001 ppb (1 pptr). Information concerning the actual rate of TBT detoxification in an estuary such as the Chesapeake bay is still lacking. Many factors come into play which could require a complex model.

4-5 TRIBUTYLTIN IN THE HUMAN FOOD CHAIN.

An intent of this study was to shed some light on the extent to which contaminated seafood contributed TBT to the human food chain. Rappahannock River oysters have traditionally been praised as some of the more palatable in the bay area. Assuming an average TBT concentration of 400 ppb (dry weight) and an average weight of 1.5 g (dry weight) per oyster, eating a dozen oysters one would ingest about 7.2 ug of TBT. Schweinfurth and Gunzel (1987) established the Acceptable Daily Intake (ADI) of TBT for an adult person to be 3.2 ug/kg body weight. The ADI for a person weighing 75 kg would therefore be 240 ug,

which is well below the 7.2 ug gained by eating a dozen oysters. One would need to ingest 400 oysters to reach this critical level. Provided the ADI is accurate, oysters are likely a negligible source of TBT toxicity for humans.

4-6 TRIBUTYLTIN FATE IN THE CHESAPEAKE BAY.

Using this report as a baseline study, it will now be possible to monitor the fate of TBT in the Chesapeake Bay. A steady decline in TBT concentration should be seen. However, considering the fact that the Chesapeake bay is a complex system with TBT contaminated sediments and still some boats painted with the compound, it may be difficult to correctly predict the actual decrease in TBT over the next few years. Future studies may shed more light on this topic.

BIBLIOGRAPHY

Alzieu C., Heral M., Thibaud J., Dardignac M.J., et Feuillet M. Influencedes Peintures Antisalissures a Base d'Organostanniques sur la Calcification de Coquille de l` Huitre. Rev. Trav. Inst. Peches marit. 45-2:101-116; 1982.

Alzieu C., Thibaud Y., Heral M. et B. Boutier. Evaluation des Risques Dus a l'Emploi des Peintures Anti-salissures dans les Zones Chonchylicoles. Rev. Trav. Inst. Peches marit. 44-4:301-48; 1980.

Alzieu, C. and Heral H. Ecotoxicological Effects of Organotin Compounds on Oyster Culture. Ecotox. testing for the Marine Environ. 2:187-196; 1984.

Beaumont, A.R. and Budd M.D. High Mortality of the Larvae of the Common Mussel at Low Concentration of Tributyltin. Mar. Poll. Bull. 15:402-405; 1984.

Becerra-Huencho, R.M. Effect of Organotin and Copper Sulfate on the Late Development and Presettlement Behavior of the Clam. Masters thesis, University of Maryland, 83pp, 1984.

Bennett, R.F. and Zedler R.J. J. Oil Col. Chem. Assn. 49:928, 1966.

Blunden, S.J., Hobbs, L.A. and Smith, P.J. The Environmental Chemistry of Organotin Compounds, In: Environmental Chemistry, H. J. M. Bowen ed., Royal Society of Chemistry, London, 1984, pp.51-77.

Donard, O.F.X., Rapsomanikis, S. and Weber J.H. Speciation and Determination of Tin and Organotin Compounds using Hydride Generation and Electrothermal Quartz Furnace Atomic Adsorption Detection. Anal. Chem., 55:2210-18, 1983.

Evans, C.J. and Laughlin R. Accumalation of Bis(tributyltin) Oxide by the Mud Crab, R. harrisii. Chemosphere, 13-1:213-19, 1984

Evans, C.J., and Karpel S. Organotin Compounds in Modern Technology. J. of Organometallic Chemistry Library, 16:1-275, 1985.

Gilmour, C.C., Tuttle J.H., and Means J.C. Determination of Picogram Quantities of Methyltinsin Sediment. Anal. Chem., 58:1848-52, 1986.

Haven, D.S., Hargis, W.J. and Kendall, P.C. The Oyster Industry in Virginia: Its Status, Problems and Promise. Special Report No. 168 in Applied Marine Science and Ocean Engineering of the Virginia Institute of Marine Science, Gloucester Point Va. 23062.

Hall, L.W., Bushlong S.J., Hall W.S. and Johnson W.E. (in press). Acute and Chronic Effects of Tributyltin on a Chesapeake Bay Copepod. Environ. Toxicol. Chem.

Hall, L.A. Jr. and Pinkney A.E. Acute and Sublethal Effects of Organotin Compounds on Aquatic Biota: An interpretative literature evaluation. CRC Critical Reviews in Toxicology, 14-2:159-209, 1985.

Hodge, V.F., Seidel S.L. and Goldberg E.D. Determitnation of Tin(IV) and Organotin Compounds in Natural Waters, Coastal Sediments and Macro Algae by Atomic Adsorption Spectrometry, Anal. Chem., 51-8:1256-9, 1979.

Huggett, R.J., Unger M.A. and Westbrook D.J. Organotin Concentrations in Southern Chesapeake Bay. In: Oceans 86 Proceedings, Volume 4, Organotin Symposium, pp. 1262-1265. Washington, D.C.

Laughlin, R, French W. and Guard H.E. Acute and Sublethal Toxicity of Bis(tributyltin) Oxide and its Putative Environmental Product, Tributyltin Sulfide, to Zoeae of the Mud Crab. Water, Air, and Soil Pollution, 20:69-79. 1983

Laughlin, R.B. Jr, French W. and Guard H.E. Accumulation of Bis(tributyltin) Oxide by the Marine Mussel Mytilus edulis. Environ. Sci. Technol, 20: 884-890, 1983.

Laughlin, R.B. Jr, and Linden O. Fate and Effects of Organotin Compounds. Ambio 14-2:88-94, 1985.

Laughlin, R.B, Nordlund K. and Linden O. Long-term Effects of Tributyltin Compounds on the Baltic Amphipod, Gammarus oceanicus. Marine Evironmental Research 12:243-71, 1984

Lee, R.F. Metabolism of Bis(tributyltin) Oxide by Crabs, Oysters, and Fish. Marine Environ. Res 17:145-8, 1985.

Maguire, R.J. Butyltin Compounds and Inorganic Tin in Sediments in Ontario. Env. Sci. Technol 18-4:291-4, 1984.

Maguire, R.J, and Tkacz R.J. Comparison of Flame Photometric and Atomic Absorption Spectrophotometric Detectors. J. of Chromatography 268:99-101, 1983.

Maguire, R., Chau Y., Bengert G., and Hale E. Occurrence of Organotin Compounds in Ontario Lakes and Rivers. Env. Sci. Tech 16-10:698-704, 1982.

Maguire, R.J, Tkacz R., Chau Y.K., Bengert G.A. and Wong P.T.S. Occurrence of Organotin Compounds in Water and Sediment in Canada. Chemosphere, 15-3:253-74, 1986.

Maguire, R.J, Tkacz R., and Sartor D.L. Butyltin Species and Inorganic Tin in Water and Sediment of the Detroit and St. Clair Rivers. J. Great Lakes Res 11-3:320-7, 1985.

Matthias, C.L; Olson G.J.; Brinkman, F.E.; and Bellama, J.M. A Comprehensive Method for the Determination of Aquatic Butyltin and Butylmethyltin Species at Ultra-trace Levels using Simultaneous Hydridization/Extraction with GC-FPD. 189th Nat'l Meeting of Am. Chem. Soc. 1985.

Matthias, C.L., Bushlong S.J., Brinckman F.E., Bellama J.M., and Hall L.W., Jr. (in press). Simultaneous Butyltin Evaluations in the Microlayer, Water Column and Sediment of a Northern Chesapeake Bay Marina.

Mueller, M.D. Tributyltin Detection at Trace Levels in Water and Sediments Using GC with FPD and GC/MS. Fresenius Z. Anal. Chem 317:32-6, 1984.

Neuman, W.P. The Organic Chemistry of Tin. 1st ed. Interscience Publishers (John Wiley & Sons) New York, 1970.

Paul, J.D, and Davies L.M. Effects of Copper and Tin Based Anti-Fouling Compounds on the Growth of Scallops Pectens maximus and Oysters C. gigas. Aquaculture 54:191-203, 1986.

Rexrode, M. Ecotoxicity of Tributyltin. In: Oceans 87 Proceedings, Volume 4, Organotin Symposium, pp. 1421-1431. Washington, D.C.

Rice, C., Espourteille F.A. and Huggett R.J. Analysis of Tributyltin in Estuarine Sediments and Oyster Tissue, Crassostrea virginica. J. of Organometallic Chemistry 1:541-544, 1987.

Roberts, M.H. Acute Toxicity of Tributyltin Chloride to Embryos and Larvae of Two Bivalve Molluscs, Crassostrea virginica and Merceneria merceneria virginica and M. merceneria. Bull. Environ. Contam. Toxicol. (in press).

Saxena, A.K. Organotin Compounds: Toxicology and Biomedicinal App.lications. Lehrstuhl II fur Anorganische Chemie 1: 39-56, 1986.

Schweinfurth, H.A. and Gunzel P. The Tributyltins: Mammalian Toxicity and Risk Evaluation for Humans. In: Oceans 87 Proceedings , Volume 4 , Organotin Symposium, pp. 1421-1431. Washington, D.C.

Smith, P.J. Structure/Activity Relationships for Di- and Tri- organotin Compounds. Int´l Tin Research Institute, 569 pp. 1978.

Stang , P.M. Butyltin Compounds in the Sediment of San Diego Bay, California. Master Thesis, San Diego State University, San Diego, California, 1986, pp 61.

Steinhauser, K.G., Amann T., Spath A. and Polenz A. Untersuchungen zur aquatischen Toxizitat zinnorganischer Verbindungen. Sonderdruck aus Vom Wasser 65, 1985

Thain, J.E, and Waldock M.J. The Effect of Suspended Sediments and Bis(tributyltin) Oxide on the Growth of Crassostrea gigas Spat. Int´l Council Expl. of the Sea, E:10, 1983.

Thain, J.E,and Waldock M.J. The Growth of Bivalves Spat Exposed to Organotin Leachates from Antifouling Paints. Int´l Council for the Expl of the Sea, E:28, 1985.

Tsuda, T., Nakanishi H., Morita T. and Takebayashi J. J. Assoc. Off. Anal. Chem. 69:981, 1986.

U´Ren, S. Acute Toxicity of Bis(tributyltin) Oxide of a Marine Copepod. Mar. Pol. Bull. 14-8:303-306, 1983.

Unger, M., MacIntire W.G., Greaves J. and Huggett R.J. GC Determination of Butyltins in Natural Waters by Flame Photometric Detection of Hexyl Derivatives with Mass Spectrometric confirmation. Chemosphere 15:461-470, 1986.

Valkirs, A.O, Seligman P., Stang P., Homer V., Liebermann S.H., Vafa G., and Dooley C.A. Measurements of Butyltin Compounds in San Diego Bay. Mar. Poll. Bull. 1985.

Westbrook, D.J., Travelstead E.J., Espourteille, F.A., Rice C.D. and Huggett, R.J. Tributyltin in Whole Water and Sediment Collected from Marinas and the Hampton Roads Area in the Southern Chesapeake Bay. Final Report to the Virginia Water Control Board. College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, Va. 1986.

Zar, J.H. Biostatistical Analysis. Second Edition. Prentice-Hall, Inc. Ed. Englewood Cliffs, N.J. 1984

VITA

François André Espourteille

Born in Paris, France on November 3rd, 1960. Graduated in June 1979 from Escola Pan Américana da Bahia, Salvador, Brazil.

Graduated from the College of William and Mary in Virginia in December 1983 with a B.S. in Biology and a minor in French.

Entered the Virginia Institute of Marine Science, College of William and Mary, in January 1984. Received a first graduate assistantship in the Department of Biological Oceanography from September 1984 to August 1985 and a second graduate assistantship in the Department of Chemical Oceanography on January 1986.

After graduation, was accepted to Rutgers, the State University of New Jersey, for doctoral studies.